SCIENTIFIC VALIDATION OF SIDDHA DRUG *"KADUKKAI VADAGAM"* FOR ITS BRONCHODILATOR, ANTI-HISTAMINE AND ANTI-OXIDANT PROPERTIES IN RODENTS

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **Scientific Validation of Siddha Drug *"Kadukkai Vadagam"* for its Bronchodilator, Anti-Histamine And Anti-Oxidant properties in Rodents** is a bonafide and genuine research work carried out by me under the guidance of **Dr. R. Karolin Daisy Rani M.D(s),** Post Graduate Department of *Gunapadam*, Government Siddha Medical College, Arumbakkam, Chennai-106 and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

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CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **Scientific Validation of Siddha Drug *"Kadukkai Vadagam"* for its Bronchodilator, Anti-Histamine And Anti-Oxidant properties in Rodents** is submitted to the Tamilnadu Dr. M.G.R. Medical University in partial fulfillment of the requirements for the award of degree of M.D (Siddha) is the bonafide and genuine research work done by **Dr A. Kalpana** Under my supervision and guidance and the dissertation has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or other similar title.

Date : **Signature of the Guide**

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ENDORSEMENT BY THE HOD,

PRINCIPAL / HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled **Scientific Validation of Siddha Drug *"Kadukkai Vadagam"* for its Bronchodilator, Anti-Histamine And Anti-Oxidant properties in Rodents** is a bonafide work carried out by **A. Kalpana** under the guidance of **Dr. R. Karolin Daisy Rani M.D(S),** Post Graduate Department of Gunapadam, Government Siddha Medical College, Arumbakkam, Chennai - 106.

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ABBREVIATIONS

ACh	Acetylcholine
ALT	Alanine Amino Transferase
ANOVA	Analysis Of Variance
AMP	Adenosine Monophosphate
AOM	Azoxymethene
AST	Aspartate aminotransferase
BHT	ButylatedHydroxy Toluene
BPT	Bronchial Provocation Testing
cAMP	Cyclic Adenosine Monophosphate
CD4+	Cluster of Differentiation 4
CMC	Carboxy Methyl Cellulose
COPD	Chronic Obstructive Pulmonary Disease
DC	Differential count
DPPH	1-Diphenyl-2-Picryl-Hydraoxzyl
E	Eosinophil
ED_{50}	Effective Dose
EDTA	Ethylene diamine tetra acetic acid
ESR	Erythrocyte Sedimentation Rate
FEV	Forced Expiratory Volume
FTIR	Fourier Transform Infrared Spectroscopy
FVC	Forced Vital Capacity
GOT	Glutamate Oxaloacetate Transaminase
GPT	Glutamate Pyruvate Transaminase

GM-CSF	Granulocyte Macrophage-Colony Stimulating Factor
Hb	Haemoglobin
HL	Human Leukemic cell lines
HPTLC	High Performance Thin Layer Chromatography
IAEC	Institutional Animal Ethical Committee
IL	Interleukin
LTC_4	Leukotriene C4
MTP	Mitochondrial Permeability Transition
MCV	Mean Corpuscular Volume
NSAID	Non-Steroidal Anti-Inflammatory Drugs
OECD	Organisation for Economic Co-Operation Development
PCT	Pre-Convulsion Time
PCV	Packed Cell Volume
PEFR	Peak Expiratory Flow Rate
PGE2	Prostaglandin E2
KV	Kadukkai Vadagam
TNF	Tumour Necrosis Factor
TLC	Thin layer chromatography
SEM	Scanning Electron Microscope
UV	Ultra violet
WHO	World Health Organization

1. INTRODUCTION

The first thing we do for ourselves after birth is to breathe. This connects us to the outside world. Life process carried out by living beings through involving the capture of oxygen from the environment and the expulsion of carbon dioxide produced in the cells. This vital process is known as breathing. It also plays an important role in the regulation of blood pH, a process known as acid-base balance and maintenance of metabolic process of cellular respiration.

When this vital breathing tract (respiratory tract) is particularly exposed to pollutants, cigarette smoke as well as ozone and other free radicals affects the natural mechanism of filtering and clearing of such pollutants through respiratory epithelium, trachea bronchial lymphatic and alveolar macrophages result in the progression of respiratory disease known as bronchial asthma. The results of recent epidemiological studies show that pollution peaks often coincide with an increase in the frequency of asthma attacks.

Asthma is a disease of airways that is characterised by increased responsiveness of the trachea bronchial tree to a variety of stimuli resulting in widespread spasmodic narrowing of the air passages which may be relieved spontaneously or by therapy. Asthma is an episodic disease manifested clinically by paroxysms of dyspnoea, cough and wheezing. However, a severe and remitting form of the disease termed status asthmatics may prove fatal. [1]

Between 100-150 million people suffering from asthma worldwide, which deaths from this condition have reached over 1,80,000 annually. India has on estimated 15-20 million asthmatics. [2]

The human economic burden associated with asthma are estimated to exceed those of TB and HIV /AIDS combined. It is also the only chronic disease with morbidity and mortality has increased over the last twenty five years.

The health effects of patients with severe asthma are very stressful. This is especially true for young asthmatics who are often severely limited in their quality of life. Currently, the main types of drugs used to treat asthma are bronchodilators and anti-inflammatory agents, which relieve symptoms of bronchospasm and reduce

inflammation of the airways. However, therapy with these drugs is not completely effective and result in adverse effects.

However, a proportion of patients with severe asthma variants required additional drugs to get the disease under control. Often required long-term moderate to high doses of oral glucocorticoids. Steroids are even inhaled in treatment which exhibit lot of side effects even at low doses like infection due to yeast in the mouth, hoarseness of voice, bone loss and glaucoma etc., which makes the condition of the patient even worse. [3]

Hence, lack of safe drugs and limited access to minimum income people used treatments stimulate the search for new substances potentially useful in the treatment of asthma. Therefore, there is no doubt promising therapies are necessary and important. Nowadays researchers have been trying for years to develop new therapeutics and therapeutic methods from natural sources especially Siddha system of medicines to treat asthma successfully.

Natural products of herbal origin, represent great pharmacological potential for asthma, because they can provide various molecules with specific mechanisms for the treatment and control of disease. The search for more effective and specific to the process for asthma shows that the demand for Siddha herbal products is promising and has an important role in the discovery of new effective and less toxic therapies for asthma.

The discovery of the connection between plants and health is responsible for the beginning of a new generation of treatments, including drugs derived from plants, the use of the plant itself or its parts, supplementary diets and functional foods. Several drugs with asthma action were isolated from medicinal plants for respiratory problems, such as atropine, theophylline and chromoglycates. Therefore, the Siddha system of medicine is still a valuable source of new anti-asthma therapeutics.

Today, nature has distinguished itself as a source of new active agents for a wide variety of diseases. In the past 25 years, including new drugs approved, approximately 30% are natural products or their derivatives.

Natural products, including plants, animals and minerals have been the basis of treatment of human diseases. History of medicine dates back practically to the existence of human civilization. Historically, the majority of new drugs have been generated from natural products (secondary metabolites) and from compounds derived from natural products. [4], [5]

According to Siddha, the five winds abiding in particular locations keep the living being going. They are Prana, Udana, Samana, Viyana, Abana .The wind moving in the mouth or face is called "Prana" "breathing forward". It upholds the body. It cause the food to enter and supports the other forces of the like- breath of the body. When upset it causes respiratory disorders include asthma and similar afflictions. [6]

In Siddha system, bronchial asthma can be compared with Eraippu irumal and also termed as Swasa kasam. It also portrays that the humour "kabam" is the cause for *"Swasa kasam".*

"கபத்தினையன்றிக் காசசுவாசங் காணாது"

- பிணிகளின் முதற் காரணம்[7]

There are so many herbal, herbo-mineral formulations are available in Siddha system of medicine to treat asthma effectively. One of the poly herbo mineral preparation *'Kadukkai Vadagam*' has been recognized as an effective treatment asthma mentioned in Siddha classical literature *"Athmaratchamirtha Vaidhiya Saara Sangiragam (part -2)"*. It is used with increasing frequency in recent years. But this drug formulation is not yet validated scientifically.

Hence, an attempt was made to find out the good possibilities of therapy and hope for further good clinical research in this area, *Kadukkai Vadagam* was selected for my dissertation topic to validate its therapeutic potential and safety profile through various physic-chemical, biochemical and instrumental analysis including toxicological and pharmacological screening.

2. AIM AND OBJECTIVES

Aim

Researchers have been trying for years to develop new therapeutics and therapeutic methods from natural sources to treat asthma. This disease needs a prolonged treatment with safe medication and quality living. This could be achieved by Siddha system of medicine as it not only treats the disease but also brings the overall wellbeing of the human.

According to the Siddha literature Kadukai vadagam was used for bronchial asthma. Thus the aim of this dissertation is to establish the Scientific Validation of the Bronchodilator, Anti-Histamine and Anti-Oxidant property of ***Kadukkai Vadagam*** for Bronchial Asthma.

Objectives

The following methodology was adopted to evaluate the safety and efficacy of the test drug in this study

- Collection of various Siddha and modern literature relevant to the study.
- Preparation of the drug according to the classical Siddha literature.
- Physicochemical and phytochemical investigation of the test drug.
- Evaluate bio-chemical analysis of the test drug to derive acidic and basic radicals.
- To estimate the percent of elements, functional groups and particle size through instrumental analysis of the trial drug.
- Evaluation of the Acute and 28 days repeated dose Toxicity of test drug according to OECD guidelines.
- Evaluation of pharmacological study of the drug through the following activities
    - Evaluation of Bronchodilator activity
    - Evaluation of Anti- histamine activity
    - Evaluation of Anti -oxidant activity of ***Kadukkai Vadagam***
- To scrutinize all the above studies to establish the potency of ***Kadukkai Vadagam***

3. REVIEW OF LITERATURE

3.1. Drug Review

The trial drug *"Kadukkai Vadagam"* was taken from the Siddha literature "*Athmaratchamirtha Vaidhiya Saara Sangiragam* (part 2)'' for treating Bronchial Asthma. The ingredients of the drug are,

- Kadukkai *(Terminalia chebula)*
- Kalluppu*(sodium chloride)*
- Korai kizhangu *(Cyperus rotandus)*
- Korochanni omam *(Hyoscyamus niger)*
- Chukku *(Zingiber officinale)*
- Kodivelli Ver *(Plumbago indica)*
- Thippilli *(Piper longum)*
- Chevuiyam *(Piper nigrum)*
- Thippilli molam *(Piper longum root)*
- Milagu *(Piper nigrum)*
- Induppu *(Sodium chloride impura)*
- Omam *(Carum copticum)*

Associated drugs

- Inji charu *(Zingiber officinale)*
- Ezhumichai charu *(Citrus limon)*
- Butter Milk

3.1.1. Gunapadam Aspect

Kadukkai

Botanical name **:** *Terminalia chebula*

Synonyms **:** *Anthan, Abhayan, Amudham, Devi, Divya, Rohini, Ammai, Abaranam, Aritaki, Varikkai, Jeevandhi*

Vernacular names

Tamil : Kadukkai

English : Myrobalan

Hindi	:	Harre, Harad, Harar
Sanskrit	:	Haritaki, Abhaya, Kayastha, Siva, Pathya
Telugu	:	Karaka, Karakkaya
Kannadam	:	Alalekai

Other Varieties **:** Visayan, Arokini, Prithivi, Amrita, Sivanthi, Thiruvirti, Abayan

Part used **:** Dried fruit

Properties

Suvai (Taste)	:	Thuvarppu, Inippu, Kaarppu, Kaippu, Pulippu
Thanmai (Nature)	:	Veppam
Pirivu (Bio- Transformation):		Kaarppu

Actions

- Digestive
- Expectorant
- Laxative
- Appetizer
- Nutrient

General characters

"தாடை கழுத்தக்கி தாலு குறியிவிடப்
பீடை சிலிபதமுற் பேதிமுடம்- ஆடையெட்டாத்
தூலமிடி புண்வாத சோணிகா மாலையிரண்
டாலமிடி போம்வரிக்கா யால்"

- அகத்தியர் குணவாகடம்.

Indications

It cures Jaw, neck, cheek diseases, Filariasis, Diarrhoea, Obesity, Rheumatoid Arthritis, Jaundice. [8]

Therapeutic uses

- *Terminalia chebula* is used in Asthma, Fever and Urinary diseases.
- Used as a gargle in sore mouth and Stomatitis, spongy and ulcerated gums.
- *Terminalia chebula* is made into a paste by adding some water and is mixed with castor oil and applied over the burns and scalds[9].

Kallupu

Chemical Name: *Sodium chloride*

Synonyms : *Kadar kuruvi, Anna koormai, Arusuvai saathi, Uvaruppingunam.*[10]

General characters

"ஐயமறுஞ் சூலை யரோசிபித்தஞ் சத்தியொடு
வெய்யபிணி யட்டகுன்மம் விட்டேகும்- பெய்வளையே
வாதமதி தாகம் மலக்கட்டும் போமுலகிற்
கோதறுகல் லுப்பைக் கொடு".

– பதார்த்தகுண சிந்தாமணி [10]

Indications

It is effective in the treatment of Kapha, Pricking pain, Loss of appetite, Pitha diseases, Eight types of Ulcers, Vatha diseases, Polydipsia and Constipation.

Korai kizhangu

Botanical name : *Cyperus rotundus*

Synonyms : *Muthakasu*

Vernacular names

Tamil	-	Korai
English	-	Nut grass
Telugu	-	Tungamuste
Malayalum	-	Muththanna
Kannadam	-	Tangahullu
Sanskrit	-	Mutha

Part used

- Rhizome

Actions

- Astringent
- Stimulant
- Tonic
- Diuretic
- Diaphoretic

- Demulcent
- Emmenagogue
- Vermifuge.

General properties

"சீத சுரந்தீர்க்குஞ் செம்புனல்பித் தம்போகும்
வாத சுரந்தணிக்கும் வையகத்தில்- வேதைசெய்ய
வந்த பிணியையெல்லாம் வாட்டுமுத் தக்காசு
கொந்துலவும் வார்குழலே! கூறு

அதிசாரம் பித்தம் அனற்றாகம் ஐயங்
குதிவாதஞ் சோபங் கொடிய- முதிர்வாந்தி
யாரைத் தொடர்டந்தாலும் அவ்வவர்க்கெ லாங்குளத்துக்
கோரைக் கிழங்கைக் கொடு" .

- அகத்தியர் குணவாகடம்

Indications

It cures Fever with rigor, Hypertension, Thirst, Delirium, Diarrhoea, Mental illness, Pitha induced thirst, Kapha disease, Calcaneal spur and Vomiting.

Therapeutic uses

- Powdered korai kizhangu taken as rejuvenative purpose for tuberculosis.
- Decoction of korai kizhangu used for diarrhoea, ulcer and vomiting.
- Tender korai kizhangu is made into paste and applied over the breast for galactogogue action.
- Preparations made out of korai kizhangu is used in childhood kapha diseases.[8a]

Korochanni Omam

Botanical name **:** *Hyoscyamus niger*

Synonyms **:** *Thipiyam, Karapi, Karsavai*

Vernacular names

Tamil	:	*Korochanni omam*
English	:	Black henbane
Hindi	:	Khorasani-Ajowan
Sanskrit	:	Parasikayavani

Telugu : Kurasani oamamu

Kannadam : Kurasani voma

Part used : Seeds

Properties

Suvai (Taste) : Kaarpu, Sirukaippu

Thanmai (Nature) : Veppam

Pirivu (Bio- Transformation) : kaarpu

Actions

- Hypnotic
- sedative
- Anodyne
- Antispasmodic
- Mild diuretic

General characters

"வெகுமூத் திரம்வாதம் வீரியநட் டம்புண்
உகுபேதி யுட்கடுப்பி னோடே-மிகுகரப்பான்
தீராக் கபமிவைபோம் செய்யகு ரோசானியென்றால்
வாரா மயக்கமுற மால்".

- அகத்தியர் குணவாகடம்.

Indications

It cures dental diseases, Amenorrhea, Dysmenorrhoea, Asthma, Rigor, Insomnia, Ulcer, Diarrhoea, Dysentery, Eczema, Kapha disease.

Therapeutic uses

- 65-320mg hyoscyamus extract is used to treat delirium, insomnia.
- It has febrifuge action. It reduces the irritation present in colon and lungs.[8b]

Chukku

Botanical name: *Zingiber officinale*

Synonyms: *Nagaram, Atagam, Aartharagum, Chonndi, Chowpannaum, Verkombu, Nava suru, Ullarntha inji, Vidam moodiya amirtham.*

Vernacular names

Tamil	:	Chukku
English	:	Dried ginger
Telugu	:	Sonti
Malayalam	:	Shukka
Kannadam	:	Ona shunti or Sunti
Sanskrit	:	Nagaram
Hindi	:	Sonth

Part used **:** Dried Rhizome

Properties

Suvai (Taste)	:	Kaarppu
Thanmai (Nature)	:	Veppam
Pirivu(Bio-Transformation)	:	Kaarppu

Actions

- Stimulant
- Stomachic
- Carminative

General Characters

"சூலைமந்தம் நெஞ்செரிப்பு தோடமேப் பம்மழலை
மூலம் இரைப்பிருமல் மூக்குநீர் - வாலகப
தோடமதி சாரந் தொடர்வாத குன்மநீர்த்
தோடம்ஆ மம்போக்குஞ் சுக்கு."

- அகத்தியர் குணவாகடம்

Indications

Dried ginger was used for Indigestion, Gastric irritation, Anal diseases, Asthma, Cough, Diarrhoea, Sinusitis, Anaemia and Fever.

Therapeutic uses

- A pinch of dried ginger powder with cow's milk is useful in loss of appetite.
- Dried ginger powder with sugarcane juice reduces burning sensation of the stomach.

- Dried ginger with sugar candy powder taken with tender coconut in morning and evening for dyspnoea and chest pain after heavy working.
- Dried ginger decoction is useful for poisonous type of fever.
- Chewing a piece of dried ginger helps in relieving the tooth ache.[12]

Kodi veli Ver

Botanical name: *Plumbago zeylanica*

Synonyms **:** *Eri, Azhal, Oli, Anichal, Vanni, Chithiram, Akni, Kodivanni, Elunaa, Vannipariyam, Karunagam, Thikku, Thisainaa.*

Vernacular names

Tamil	:	Kodivelli
English	:	Ceylon lead-wort
Hindi	:	Chita,chitra
Sanskrit	:	Angi-shika
Telugu	:	Tella-chitra-mulam
Kannadam	:	Chitra-mula

Part used : Root

Properties

Suvai (Taste)	:	Kaarpu
Thanmai (Nature)	:	Veppam
Pirivu (Bio- Transformation)	:	Kaarpu

Actions

- Anti-periodic
- Diaphoretic

General characters

"கட்டிவிர ணங்கிரந்தி கால்கள் அரையாப்புக்
கட்டிச்சூ லைவீக்கங் காழ்மூலம்- முட்டிரத்தக்
கட்டுநீ ரேற்றம் கனத்த பெருவயிறும்
அட்டுங் கொடிவேலி யாம்"

- அகத்தியர் குணவாகடம்.

Indications

It cures Tumour, Ulcer, Dropsy, Piles, Sinusitis, Ascites, Abscess, Pricking Sensation, Haemorrhoids, Syphilis, Filarial Fever and Septic fever.

Therapeutic uses

- A paste formed from kodiveli with gingely oil and is applied over Piles, Inguinal bubo and Cervical lymph nodes. It also used to dilate the cervix.
- A paste formed by grinding the root with milk is used to reduce the effect of poisons.[8c]

Thippili

Botanical name: Piper longum

Synonyms **:** *Pippli, Aadhi,Kaaman, Sowndi, Kanam, Saram, Koli, Ambu, Aathimarunthu, Kanai.*

Vernacular names

Tamil	:	Thippili
English	:	Long pepper
Telugu	:	Pippilu
Malayalam	:	Thippili
Kannadam	:	Hippili
Sanskrit	:	Pippali
Hindi	:	Pipar

Part used : Dried fruit and Roots

Properties

Suvai (Taste)	:	Kaarppu
Thanmai (Nature)	:	Veppam
Pirivu (Bio- Transformation)	:	Kaarppu

Actions

- Carminative
- Stimulant
- Expectorant

- Antiseptic
- Febrifuge

General characters

"கட்டி யெதிர்நின்று கடுநோயெல் லாம்பணியும்
திட்டி வினையகலும் தேகமெத்த-புட்டியாம்
மாமனுக்கு மாமனென மற்றவர் மற்றவனாங்
காமமெனுந் திப்பிலிக்கும் கை".

- தேரன் வெண்பா

Indications

It relieves Kapha related diseases and strengthens the body. [8d]

Therapeutic uses

- Powered long pepper with honey will relieve cough, cold, asthma, hoarseness and hiccough.
- Long pepper powder with honey and betel leaf juice cures fever.
- A mixture of long pepper, long pepper root, black pepper and ginger in equal proportions is used to relieve colic and flatulence.
- Powered form of long pepper seeds with ghee is used for its aphrodisiac action. [12a]

Chevuiyam (Milagu Ver)

Botanical name **:** *Piper nigrum* (Black pepper root)

Synonyms **:** *Kandirai, Savigai, Saviyam*

Vernacular names

Tamil	:	Milagu
English	:	Black pepper
Telugu	:	Miriyalu
Malayalum	:	Kurumilagu
Sanskrit	:	Maricha
Hindi	:	Kali-mirch

Part used **:** Root

Properties

Suvai (Taste)	**:**	Kaippu, Kaarppu
Thanmai (Nature)	**:**	Veppam
Pirivu (Bio- Transformation)	**:**	Kaarppu

Actions

- Carminative
- Stimulant
- Anti-vadha
- Antidote

General characters

"சூலை அருகிசன்னி தொல்லிருமல் ஈளைபித்தம்
மேலைக் குரற்கம்மல் வெங்களநோய் -மூலசுரம்
கவ்வியங்கத் தேறு கனதா வரவிடமுஞ்
செவ்வியங் கொள்ளவிடுந் தேர்"

- அகத்தியர் குணவாகடம்.

Indications

It cures spasmodic pain, Tastelessness, Chronic fever, Chronic Cough, Hoarseness, Throat diseases, Fever.

Therapeutic uses

Oil prepared from Cheviyum is used for catarrh. [8e]

Thipilli Moolam

Botanical name **:** *Piper longum (long pepper root)*

Synonyms **:** *Ampinati, Kiranthinadi ver, Kiranthigam, Thippili ,Thippili kattai, Nathikaranthai, Narukku vaeru, Narukku thippili, Kanda thipppili, Modi ver.*

Vernacular names

Tamil	:	*Thippili moolam*
English	:	Long pepper- root
Telugu	:	*Pippili-mulam*
Malayalam	:	*Kattu thippili*

Kannadam : *Hippfli-beru*

Sanskrit : *Pipalee-moola*

Hindi : *Felfelai-maya*

Part used : Roots

Properties

Suvai (Taste) : *Kaarppu*

Thanmai (Nature) : *Veppam*

Pirivu (Bio- Transformation) : *Kaarppu*

Action : Stomachic

General characters

"தாகபித்தஞ் சோகந் தணியாச் சுரமிருமல்
மேகங் குரற்கம்மல் மெய்க்கடுப்பும் - ஏகுங்காண்
திப்பிலிமூ லங்கண்ட்த் திப்பிலிய தாம்நறுக்குத்
திப்பிலியென் றேயொருக்காற் செப்பு".

- அகத்தியர் குணவாகடம்

Indications

It cures Puerperal fever, Cough, Syphilis, Hoarseness, Burning sensation, Chronic fever, Diarrhoea and Body pain.

Therapeutic uses

- A paste formed by grinding the root with milk is used to treat thirst, back pain and vatha disease.
- Root powder is used as inhaler for angina and Syncope. [8f]

Milagu

Botanical name: *Piper nigrum*

Synonyms : *Malayali, Masam, Sarumabandam, Kayam, Kalinai, Miriyal.*

Vernacular names

Tamil **:** Milagu

English **:** Black pepper

Telugu **:** Miriyalu

Malayalam	:	Kurumilagu
Sanskrit	:	Maricha
Hindi	:	Kali-mirch

Part used : Dried fruit

Properties

Suvai (Taste)	:	Kaippu, Kaarppu
Thanmai (Nature)	:	Veppam
Pirivu(Bio- Transformation)	:	Kaarppu

Actions

- Carminative
- Stimulant
- Anti-vadha
- Antidote

General characters

"சீதசுரம் பாண்டு சிலேத்மங் கிராணிகுன்மம்
வாதம் அருசிபித்தம் மாமூலஓது - சன்னி
யாசமபஸ் மாரம் அடன்மேகம் காசமிவை
நாசங் கறிமிளகினால்".

- அகத்தியர் குணவாகடம்

Indications

It cures Anaemia, Gastric ulcer, Giddiness, Diarrhoea, Dysentery, Vomiting, Anal fissure and Cataract. [8g]

Therapeutic uses

- Dried unripe fruits are prescribed in cholera, dyspepsia, flatulence and various gastric ailments.
- Powdered black pepper with onion and salt is made into a paste, and this mixture is applied on the scalp to cure alopecia and also to increase the hair growth.
- Black pepper paste is applied externally for boils.
- Powder of black pepper is used as tooth powder. [12b]

Induppu

Chemical name : *Sodium chloride impura*

Synonyms : *Sainthavam, Sinthooram, Santhiranuppu, Mathiyuppu, Mathikoormai, Minthaachchol, Sainthalavanam, Mathilavanam.*

Actions

- Laxative
- Carminative
- Diuretic
- Stomachic

General Characters

"அட்டகுன்ம மந்தம் அசிர்க்கரஞ்சூர் சீதபித்தந்
துட்டவையம் நாடிப்புண் டோடங்கள் - கெட்டமலக்
கட்டுவிட விந்தையக் காமியநோய் வன்கரப்பான்
விட்டுவிட விந்துப்பை விள்."

- குணபாடம் தாது சீவம் வகுப்பு

Indications

It cures Gastric ulcer, Constipation and Cough.

Therapeutic uses:

- As a Digestive, a compound powder made of Rock salt, *Terminalia chebula*, *Phyllanthus emblica* and Long pepper in equal parts, recommended in doses of 10 grains twice a day.
- Used in the treatment of sprains externally
- Beneficial in dyspepsia and other abdominal disorders. [13]

Omam

Botanical name : *Carum copitum*

Synonyms : *Asamotham, Thipiyam*

Vernacular names

Tamil **:** *Omam*

English **:** The bishops weed

Malayalam : Ayamodakam

Hindi : Ajvayam

Sanskrit : Yavani

Telugu : Omamu

Kannadam : Voma

Part used : Seed

Properties

Suvai (Taste) : Kaarpu

Thanmai (Nature) : Veppam

Pirivu (Bio- Transformation) : Kaarpu

Actions

- Stomachic
- Antispasmodic
- Carminative
- Antiseptic
- Stimulant
- Tonic
- Sialogogue

General characters

"சீதசுரங் காசங் செரியாமந் தம்பொருமல்
பேதியிரைச் சல்கடுப்பு பேராமல் -ஒதிருமல்
பல்லொடுபல் மூலம் பகமிவைநோ யென்செயுமோ
சொல்லொடுபோம் ஒமமெனச் சொல்"

- அகத்தியர் குணவாகடம்.

Indications

Cough, Diarrhoea, Bronchial asthma, Dental diseases. [8h]

Therapeutic uses

- Dose of the oil is from 1-3 drops on sugar or made into an emulsion with water .Externally it is applied to relieve rheumatic and neuralgic pain.
- Oil and the distilled water from the seeds, known as omam water in doses of 1 to 2 Ounces are useful in the early stages of Cholera.

- Oil and flowers of carum copitum combined with soda forms a nice remedy for acidity, dyspepsia and flatulence. [14]

Butter milk

It is a food habit in Tamilnadu to take rice with butter milk at the end of a meal. It prevents constipation and reduces body heat. It also acts as a mild diuretic, reduces pain and swelling of the ankles and makes free maturation. Cow's buttermilk is better for heavy walkers and hungry persons and persons who are suffering from heat. It cures heat and thirst.

For patient suffering from leprosy, butter milk is more useful during medication, which eliminates toxins along with medicines in the urine. Butter milk is used as a best adjuvant and regimen in the diseases of ascites. [13a]

Elumichai

Botanical name : *Citrus limon*

Vernacular names

Tamil : Elumichai
English : Lime
Malayalam : Cheru-naranga
Hindi : Ninbu limu
Sanskrit : Jambira
Telugu : Nimma
Kannadam : Nimbe

Part used: Leaf, fruit, fruit juice, oil.

Properties

Suvai (Taste) : Pulippu
Thanmai (Nature) : Veppam
Pirivu (Bio- Transformation): Kaarpu

Actions

- Rubefacient
- Carminative

General characters

"தாகம் குநகநோய் தாழாச் சிலிபதநோய்
வேகங்கொள் உன்மாதம் வீறுபித்தம் -மாகண்ணோய்
கன்னனோய் வாந்தியும்போங் கட்டுவா தித்தொழிலில்
மன்னெலுமிச் சங்கனியை வாழ்த்து"

- அகத்தியர் குணவாகடம்.

Indications : Vomiting, Nausea, Dehydration, Psychotic disorder. **[8i]**

Therapeutic uses

- A pickle of this fruit is popular and effective remedy for indigestion
- Nimba thailam applied as special usage in leprotic ulcers.[14a]

Inji

Botanical name : *Zingiber officinale*

Synonyms : Allam, Artharagam, Arthiragam, Ilakottai, Narumaruppu mathil.

Vernacular names

Tamil : Inji
English : Green ginger-fresh root of-dry ginger
Malayalam : Inji
Hindi : Adarakh,sonth
Sanskrit : Adrakam
Telugu : Alluma
Kannada : Hashi-shunti,Vona-sunthi

Part used : Rhizomes

Properties

Suvai (Taste) : Kaarpu
Thanmai (Nature) : Veppam
Pirivu (Bio- Transformation) : Kaarpu

Actions

- Carminative
- Stomachic

- Sialogogue
- Digestive
- Stimulant
- Rubefacient

General characters

"இஞ்சிக் கிழங்குக் கிருமல்ஐயம் ஓக்காளம்
வஞ்சிக்குஞ் சன்னிசுரம் வன்பேதி-விஞ்சுகின்ற
சூலையறும் வாதம்போந் தூண்டாத தீபனமாம்
வேலையுறுங் கண்ணாய் விளம்பு."

- அகத்தியர் குணவாகடம்.

Indications : It cures Tuberculosis, Cough and Diarrhoea.

Therapeutic uses

- Ginger juice mixed with sugar candy and given twice daily is a good remedy for diabetes (both types – mellitus and insipidus)
- 12 ml of each ginger and onion juice is mixed together and given helps in relieving Nausea and Vomiting.
- Ginger juice rubbed on and around the Umblicus is said to cure all kinds of Diarrhoea. [8j]

3.1.2. Botanical Review

Kadukkai (*Terminalia chebula*)

Scientific classification [15]

Kingdom	:	Plantae
Class	:	Dicot
Order	:	Mytales
Family	:	Combretaceae
Genus	:	*Terminalia*
Species	:	*chebula*

Occurrence and distribution

It was distributed in chiefly in deciduous forests and areas of light rainfall, but occasionally in moist forests, up to about 1500 m throughout India. Abundant in northern India; also occurs in Bihar, West bengal, Assam.

Description of the plant

A large tree young branchlets, leaf buds and leaves with long, soft, shining, rust coloured, sometimes silvery hairs. Leaves are mostly sub-opposite distant, ovate or oblong ovate, 8 to 20 cm long, deciduous in the cold season. Flowers dull white or yellowish, with a strong offensive smell, borne in spikes from the upper axils and in small terminal panicles. Bark 6mm thickness, dark brown with shallow vertical cracks wood very hard, brownish grey with a greenish or yellowish tinge. Fruit yellowish - brown, ovoid, 20 to 55 mm long, 13 to 25 mm wide, wrinkled and ribbed longitudinally. Non adherent to the seed taste astringent.

Chemical Constituents

- Fruits and bark contains Gallic acid, Terminoic acid, Ferulic acid, Vanillic acid and Tannin.
- In leaves the characteristic compounds are Tannins, Triterpenes, saponins and mucous substances. The tannins are esters of different phenol-carbonic acids.
- The fruit of Terminalia chebula contains three hydrolysable Tannins, Chebulinic acid, Chebulagic acid. [16]

Korai kizhangu *(Cyperus rotundus)*

Scientific classification

Kingdom	:	Plantae
Class	:	Liliopsida
Order	:	Poales
Family	:	Cyperaceae
Genus	:	Cyperus
Species	:	rotundus

Description

- It is a perennial, stolaniferous, rhizomatus, halophytic sedge. Rhizome many slender,
- Tuber-white, succulent when young, hard and black when mature; stem-leafy at base arising from a tuber. Leaf above dark green, with reddish brown sheaths, clustered at the base of stem. Inflorescence 3-9 spreading rays bearing tassels of few, large spikelet; spikelet 20-40 flowered, red brown to almost black. Fruit shape is oblong ovate.

Constituents

α-cyperone, β-selinene, cyperene, patchoulenone, sugeonol, kobusone, and isokobusone, that may scientifically explain the folk- and alternative-medicine uses. A sesquiterpene, rotundone, so called because it was originally extracted from the tuber of this plant

Part used : Tubers

Medicinal Uses

The root is pungent, acrid, cooling, astringent, appetizer, stomachic, anthelmintic and useful in treatment of leprosy, thirst, fever, blood diseases, biliousness, dysentery, pruritis, pain, vomiting, epilepsy, opthalmia, erysipelas etc.[17]

Inji *(Zingiber officinale)*

Scientific classification

Kingdom	:	Plantae
Class	:	Monochlamydeae
Order	:	Zingiberales

Family : Zingiberaceae

Genus : *Zingiber*

Species : *officinale*

Occurrence and distribution

Inji is the rhizome of *Zingiber officinale* (Family: Zingiberaceae), widely cultivated in India, rhizomes dug in January- February, buds and roots removed, soaked overnight in water, decorticated and sometimes treaded with lime and dried.

Description

Macroscopic

Drug occurs as entire rhizome or in pieces, rhizome laterally compressed bearing flattish ovate, oblique branches on upper side, each having a depressed scar at its apex, pieces 5 to 15 cm. Long, 1.5 to 6.5 cm. Wide (usually 3 to 4 cm.) and 1 to 1.5 cm.thick,fracture short with projecting fibres, transversely cut surface shows a wide central stele having numerous greyish cut ends of fibres and yellow secreting cells; odour characteristic; taste pungent.

Microscopic

Rhizome – Shows a few layered, irregularly arranged, tangentially elongated, brown cells of outer cork and 6 to 12 rows of thin-walled .colourless, radically arranged cells of inner cork. secondary cortex consisting of hexagonal to polygonal, isodiametric, thin-walled, parenchymatous cells containing numerous circular to oval starch grains with characteristic striations and hilum at one end measuring 5 to 25 µm in dia., idioblasts containing large yellowish to brownish globules of oleo-resin; walls of oil cells suberised; numerous ckised, conjoint, collateral, cortical fibro-vascular bundles scattered throughout cortical zone, greater number occurring in inner cortical region, larger bundles consists of 2 to 7 vessels, small cells of sieve tube; pericycle single layered enclosing central stele; larger bundles found scattered throughout stele, composed of xylem, phloem, parenchyma and sheath of sclerenchyma.

Constituents

Volatile oil containing cineole, zingiberol, zingiberene, bisabolene and phellandrene, gingerdione, dihydro gingerol, dexahydro curcumin and desmethyl hexahydro curcumin, dehydro gingerdione. [17a]

Chukku *(Zingiber officinale)*

Chemical Constituents

α-zingiberene, gingerin, beta-sesquiphellandrene, gingerdione, gingerdiols, gingerdiacetates, 6-gingersulfonic acid, gingerol, shogaol, diterpenes, ginger glycolipids A,B & C, curcumene, fats and starch. [18]

Milagu *(Piper nigrum)*

Scientific classification [19]

Kingdom	: Plantae
Class	: Dicot
Order	: Microembryeae
Family	: Piperaceae
Genus	: *Piper*
Species	: *nigrum*

Occurrence and distribution

The plant cultivated in the hotter and moist parts of India, in evergreen forest up to 1,500 meters.

Description of the plant

Climbing perennial shrubs, rooting at the nodes, leaves are cordate or round based; flowers minute in spikes usually dioeciously. Fruiting spikes very variable in length, fruits ovoid or globose one seeded berries, bright red when ripe, seeds are globose, albumin hard and testa thin greyish-black to black, perisperm hard, wrinkled and white, 0.4 to0.5 cm in diameter; odour aromatics, taste pungent.

Flowering occurs in the rainy season and fruits ripening in the autumn season (December to April) [20].

Chemical Constituents

Piperine, chavicine, Piperettine, Piperoline A&B, Trichostachine, N-trans-feruloyl piperidine, Feruperine, Citrohellol, Arginine, Piperolic acid, Serine, Ascorbic acid, Carotene[9a] .

Milagu Ver *(Piper nigrum)*

Chemical Constituents

Piperin, piperlatin, brachyamideA and B, brachystine,sterols. [21]

Thippili *(Piper longum)*

Scientific classification [22]

Kingdom	:	Plantae
Class	:	Dicot
Order	:	Microembryeae
Family	:	Piperaceae
Genus	:	*Piper*
Species	:	*longum*

Occurrence and distribution

This plant mostly occurs in hotter parts of the India from central Himalayas to Assam up to lower hills of the west Bengal and evergreen forests of western Ghats as wild and also cultivated in north east and south. It grows in Kurinjinilam.

Description of the plant

A slender aromatic climber and leaves alternative, lower ones broadly ovate cordite, upper ones oblong, oval, all entire 5 to 7 nerved leaves; male spikes longer, slender, 2.5 to 7.5 cm long. Female spikes short, cylindrical, 1.5 to 2.5 cm long, 5 to 7 mm thick. Fruit greenish- black to black, cylindrical, 2.5 to 5 cm long and 0.4 to 1 cm thick, consisting of minute sessile fruits, arranged around an axis. Surface rough and composite; broken surface shows a central axis and 6 to 12 fruitlets arranged around an axis. Odour is aromatic; taste is pungent producing numbness of the tongue [20a].

Chemical constituents

Piperine, piperidine, Piperlongumine, Pipernonaline, Sesamin, Piperundecalidine, Futoamide, Piplasterol, Volatile oil [23] .Pellitorine, Piplartine[24].

Thippli moolam *(Piper longum)*

Chemical constituents

Piperine, sitosterol, cepharadiones. [25]

Induppu (*Sodium chloride impure)*

Chemical Formula : NaCl (impure sodium chloride)

Vernacular Names

English	:	Rock salt, Sea salt, Bay salt, Sodium chlorate
Hindi	:	*Sendhalon, sedhalon*
Malayalam	:	*Intu-Uppu*
Sanskrit	:	*Saindhava*
Telugu	:	*Saindhalavanam*
Tamil	:	*Indu-Uppu*

Characters

- Found in small white crystalline grains or transparent cubes.
- Brownish white externally and white internally.
- It is a soluble compound having a characteristic salty taste.
- Pure salt – colourless and transparent, but often variously coloured as tinged grey or blue or brown or pink due to impurities.

Specific gravity

2.1 – 2.6

Composition of Rock salt (% dry basis)

Sodium chloride	-	68.85
Manganese chloride	-	0.55
Calcium chloride	-	0.53
Magnesium chloride	-	0.43
Sodium bicarbonate	-	0.74
Insoluble matter	-	30.34
Moisture	-	1.54

Solubility

- 35.7 g/100g of water at 0ºC
- 39.8 g/100g of water at 100ºC

Actions

- Rock salt possesses stronger purgative properties than Cream of Tartar.
- Laxative – dosage : 4.2 to 8.4gm

- Purgative – dosage: 16.8 to 21gm
- Carminative
- Diuretic
- Stomachic. [26]

Kodi veli *(Plumbago zeylanica)*

Synonyms

Plumbago viscosa

Common names

Ceylon lead word, Doctor bush, Plumbago

Etymology

- The generic name Plumbago is derived from the Latin plumbum, lead.
- The specific epithet zeylanica is from the Latinized name for Sri Lanka (Ceylon).

Scientific Classification

Kingdom : Plantae
Class : Magnoliopsida
Order : Plumbaginales
Family : Plumbaginaceae
Genus : *Plumbago L.*
Species : *zeylanica*

Distribution

It is a perennial herb distributed widely in India, much cultivated widely in the peninsular regions, probably in Bengal, Malay, Peninsula, Ceylon, tropics of the world.

Description

Macroscopic Characters

Mostly perennial shrubs of about 60-120 cm in height. Leaves alternate, ovate, acute, glabrous, entire, short stalk, flowers white, bracteates, glandular and elongated spikes, 10-30 cm long. Seeds oblong shaped. Roots 30 cm or more in length, 6 mm or more in diameter as also as short stout pieces, including root stocks

reddish to deep brown, bark thin and brown, internal structure striated, odour, disagreeable, taste, acrid. [27]

Microscopic Characters

Transverse section of root shows outer most tissue of cork consisting of 5 -7 rows, secondary cortex consists of 2-3 rows of thin walled rectangular, light brown cells, starch grains. Secondary cortex followed by a wide zone of cortex is composed of large polygonal parenchymatous cells varying in size and shape, containing starch grains and some cells with yellow contents, fibres scattered singly or in groups consisting of usual elements and phloem fibres, similar to cortical zone, phloem fibres usually in groups of 2-5 or more but occasionally occurring singly, lignified with pointed ends and narrow lumen, cambium indistinct, xylem light yellow to whitish radically arranged, 1-6, seriate, radically elongated, starch grains, stone cells absent.

Constituents

Plumbagin, citranone, elliptone, ß-sitosterol-glucoside, bakuchiol, phenols, isoaffinetin, saponaretin, flavanoids, psorealen, iso-orientin. [28]

Korochanni Omam *(Hyoscyamus niger)*

Scientific classification [29]

Kingdom	:	Plantae
Class	:	Magnoliopsida
Order	:	Solanales
Family	:	Solanceae
Genus	:	Hyocyamus
Species	:	niger

Occurrence and distribution

Kashmir to garhwal, from 2,400m to 3,300m.

Description of the plant

An erect, foetid, viscidly hairy annual or biannual up to 1.5 m in height, radical leaves spreading ,coarsely dentate to pinnately lobed, stem leaves smaller, ovate, irregularly pinnatified, flowers yellowish green, veined with purple darker in the centre, lower ones in forks of the branches. Upper solitary in the axils of leaf-like

bracts, fruits capsules, enclosed in globose enlarged calyx, seeds numerous, minute, kidney shaped, brown. [30]

Chemical Constituents

Hyoscine, hyoscyamine, oleic and linoleic acids, N-trans - feruloyl tyramine, cannabsin D, cannabsin G, vanillic acid, hyoscyamide, rutin. [31]

Omam *(Carum copticum)*

Scientific classification [32]

Kingdom	**:**	Plantae
Class	**:**	Magnoliopsida
Order	**:**	Apiales
Family	**:**	Apiaceae
Genus	**:**	Carum
Species	**:**	copitum

Occurrence and distribution

Cultivated throughout India.

Description of the plant

An erect branched annual herb, stems striate, leaves 2-pinnate, ultimate segment all linear, flowers pure white in compound umbels, fruits ovoid, ultimately shining, yellow, aromatic cremocarps, mericarps with faint ridges, compressed. [33]

Chemical Constituents

Thymol, gamma- terpinene, p-cymene. [34]

Kalluppu (*Sodium chloride impure)*

Chemical Name **:** *Sodium chloride impura*

Chemical Formula **:** NaCl (impure sodium chloride)

Characters

Black salt is type of rock salt. It is also known as Himalays black salt. The condiment is composed largely of sodium chloride with several

other components lending the salt its colour and smell. The smell is due to its sulfer content. Because if the presence of iron sulfide it forms brownish pink to dark violet translucent crystals when whole and when ground into a powder it is light purple to pink in colour.

Composition of black salt

It consist primary of sodium chloride and trace impurities of sodium sulphate, sodium bisulfide, sodium sulfide, iron sulphide.

Unrefiend sea-salt contains small amounts of magnesium and calcium halides and sulphates and sulphates traces of algae products, salt resistance bacteria and sediment particle. [35]

Ezhumichai ***(Citrus limon)***

Scientific classification [36]

Kingdom	:	Plantae
Class	:	Magnoliopsida
Order	:	Sapindales
Family	:	Rutaceae
Genus	:	Citrus
Species	:	limon

Occurrence and distribution

Throughout India, cultivated in plains and hills in areas up to 1,200m elevation.

Description of the plant

A much branched thorny shrub with spreading branches, leaves unifoliately compound, rachis winged, leaflet elliptic-oblong, alternate, coriaceous, entire or crenulate, flowers white in short racemes, fruits large, globose berries with thick or thin rind, pulp pale, very acid. [37]

Chemical Constituents

Polyphenols, terpenes, tannins, citric acid. [38]

Morden aspect of buttermilk

Buttermilk is made by reintroducing lactobacillus acidophilus, the probiotic bacteria that gives buttermilk its sour taste.

Buttermilk contains a wide range of vitamins and minerals, including calcium, iron, magnesium, potassium, phosphorus, sodium and zinc. [39]

3.2. Disease Review

3.2.1. Siddha Aspect of the Disease

***Swasakaasam* (Bronchial Asthma)**

Other names

- *Iraippu*
- *Izhuppu noi*
- *Swasam*
- *Thoivu*
- *Eelai*
- *Suram*
- *Iraippirumal*

Nature of the disease

Swasakasam arises with severe chest tightness leading to difficulty in inspiration and expiration of the air (i.e. dyspnoea). In addition to difficulty in breathing while exhaling the air, expiratory noise will be produced resembling the sounds of musical instruments like flute, veena, lute etc., are heard obviously. Further if hard attempts are made to expel the phlegm, it results in vain.

Genesis of the disease

"கால்பெருக் குணவுபொருள் தண்ணீர் மாறல்
கருதிருமல் மிகல்வாந்தி குளிர்ந்த காற்று
மால்செய்து நாள்தோறும் வருந்துங் காய்ச்சல்
மந்தன முயிர்நிலை யிலடிகள் தாங்கல்
ஏல்சீத பேதிவிட பாண்டு புகைகள்
இலகிய நெல்லாதிமணிச் சுனையுட் செல்லல்
மேல்வழியிற் சிலவரினு மிரைப்பாம் நோயு
முனிவர்கள் விளம்பினாரே"

- கையெழுத்துப்பிரதி

Swasakasam is caused due to the following factors such as

- Ingestion of allergic food stuffs
- Allergy inducing activities such as exposed to cool climates.
- Immunity deprivation

- In take of diet which increases kapha.
- Grass, rice and ragi also triggers the sign and symptoms
- Symptoms may also develop due to inhalation of foul smelling substances.

Prodromal symptoms

"மார்பில் விளாவிரண்டில் மண்ணுமிகு நெரியில்
சேர்ந்து வலித்தல் திணறல் - தார்மூச்சு
உப்பல் வயிற்று லுருவது முற்குறியாச்
செப்பிரைப்பு நோய்க்குதனைத் தேர்"

- யூகி வைத்திய சிந்தாமணி[40]

Generally the prodromal symptoms and intensity of the disease will be recognised earlier by chronic asthmatics. While taking unsuitable food and while inhaling the chill air the patient develops rhinitis, sneezing, chest discomfort, chest tightness, pain, difficulty in normal breathing, pain in para vertebral region with dyspnoea, distension of abdomen and excess sweating.

Types of swasakasam

Swasakasam (Iraippu) has been classified into 5 types.

Of these, first four types are based on *kuttram* and the final one is based on intensity of breath. They are as follows

1. *Vali Iraippu*
2. *Iya Iraippu*
3. *Iyavali Iraippu*
4. *Mukkutra Iraippu*
5. *Melnokku Iraippu*

Apart from this further it is also classified into another 5 types.

"சிறுபே றிரைப்பு திணறல் மந்தாரம்
வருமே லிரைப்புந் தின்மாண்பு"

- யூகி வைத்திய சிந்தாமணி[40]

They are as follows

1. *Sittru Iraipu*
2. *Per Iraippu*

3. *Thinaral Iraippu*
4. *Mandhara Iraippu*
5. *Mael Iraippu*

Signs and symptoms

"வன்மையாய் கோழைகட்டி இருமி வீழும்
மாநாகம் போலவே வாங்குஞ் சுவாசம்
திண்மையாய்ச் சேருமலுண்டா மடிக்க டிக்குஞ்
சீரண மிலாமலே வயிறு மூதும்
நன்மையாய் நாசியது தணல்போ லாகும்
நலிந்துடம்பு வற்றி வருங் குரலுங் கம்மும்
உண்மையா யுண்ணாக் கிலுாறுங் கேணி
யுழந்துமே சுவாசகா சத்தி னொப்பே"

- யூகி வைத்திய சிந்தாமணி[40a]

Vali iraippu noi

Vatha dosha gets aggravated due to ingestion of food that is not easily digested, wandering under hot sun rays, eating tubers. Due to increased *vatha dosha* the patient may feela condition as if nothing is inside the chest. Inspite of all these conditions patient doesn't experience severe illness and this condition is curable. *Vali iraippu* is also mentioned as "*Soothira swasam".*

Kabha swasam

Kapha swasam is caused due to increased *kapha dosha* because of taking foods which increase *kapha* and also roaming in chill air. It produces nasal congestion, rhinitis, chest tightness, inability to breathe.

Sometimes constricted type of chest pain may aggravate to the extent as if the patient dies of inability to breath. When the patient mildly attempts to cough and expectorates some mucus relief occurs for some extent. When the patient does not cough and expel the mucus dyspnoea occurs and patient is unable to lie on bed, makes him to stand.

Sweating on forehead, blackening of face, chillness of limbs, dryness of tongue, shivering of body, dyspnoea, inability to sleep are the associated symptoms of this disease. It is also known as '*Thamaraga suvaasam'.*

Iyavali iraippu

In this condition both *kapha dhosam* and *vatha dhosam* are dearranged together and causes the following symptoms. Symptoms of this type will be very severe and the derangement of *vatha dosha* combines along with *udhana vaayu*. Clinical features of this type are dyspnoea, inability to inspirate and expirate air, constipation, abdominal distension, dryness of tongue, redness and painful eyes, shivering of body, giddiness, excessive sleep, incoherent talk, etc., this condition is also called as "*Vichinna Swasam".*

Mukkuttra iraippu

In this condition, all the three doshas gets deranged at once and *udhana, abanan, viyanan, samanan* get deranged one by one which in turn affects the seven major elements of the body. It is life threatening type of asthma. The prodromal symptoms are Shivering of body, dyspnoea, depression, breathing like cow's breathing, chest tightness and pain, constipation, oliguria, pain all over the body, stammering and excessive sweating over the forehead. This is also called as "*Thinaral Iraippu*".

Maelnokku Iraippu

If any of the above mentioned disease continues for many days without response to treatment, then the upward directional *udhana vaayu* loses its strength and in such a situation, expiration may not be possible. The patient tends to develop dyspnoea with prominent eyeball. There may be dryness of mouth. Patient may be unable to speak; he may appear astonished and will not lie down on the bed he may look upward he may also attempt to exhale by his opened mouth. If proper treatment is given at this stage he may survive. Otherwise he may fall unconscious with darkening of face and may die with mouth open [41].

Other factors affecting the disease

- Eating foods which will induce excessive *kapha.*
- Exposure to chill air.
- Living in the mountains.
- Walking in the cold climate.

Pulse

"கபமல்லாது காச சுவாசம் வாராது"[7]

- *Kaba Nadi*
- *Vathakaba Nadi*
- *Kapthpitha Nadi* are the classical pulse for *Swasakasam*.

Sputum

- If the sputum is found excessive in quantity, light weight and foamy, it is considered that the disease gets developed due to *Kapha dosham*.
- If the sputum is black in colour, hard and with smell of flesh, it will denote *Kapha dosham.*
- If it is found white like pus and mixed with yellow colour, it will denote P*itha dosham*[42]

3.2.2. Modern aspect of the disease

Bronchial asthma

Introduction

Asthma, the word was derived from Greek word. The term **"ASTHMA"** in Greek means breathless or breathe with open mouth.

Asthma is defined as a chronic inflammatory disorder of the airways, characterised by reversible airflow obstruction causing cough, wheeze, chest tightness and shortness of breath.

Inflammation of the bronchial wall involving eosinophil, mast cells and lymphocytes, together with the cytokine and inflammatory products of these cells, induces hyper-responsiveness of the bronchi so that they narrow more readily in response to a wide range of stimuli. Narrowing of the airway in usually reversible, but in some patients with chronic asthma the bronchial wall inflammation may lead to irreversible obstruction of airflow.

Epidemiology

The prevalence of asthma increased steadily over the later part of the century first in the developed and then in the developing countries. Current estimates suggest that asthma affects 300 million people world-wide and additional 100 million persons will be diagnosed by 2025.In India,15-20 millions are asthmatics.About 2, 50,000 annual deaths.

Epidemiological studies suggest that the multiple genetic and environmental factors contribute to the causation of asthma, a clinical condition that is viewed as a cluster of related disorders to smooth muscle hypertrophy. [43]

Factors that triggers asthma

- Smoking
- Infections like cold
- Allergens such as food, pollens, dust mites and pet dander
- Exercise
- Air pollution and toxins
- Emotional stress and anxiety
- Weather, especially extreme changes in temperature

- Drugs (such as aspirin, NSAID and beta blockers)
- Perfumes and fragrances
- Acid reflux

Allergens are the most causative factor for 50 to 70% for adults in asthma. In children under 3 years of age, viral infections (respiratory syncytial virus) are the most common trigger. After 3 years of age, the allergies also begin to play an increasing role as a trigger. After 20 years of age, occupational exposure to any toxic substances and allergens also can be important triggers.

Dietary deficiency of antioxidants may predispose to development of asthma from childhood days [44]

Pathology

- Inhaled allergens stimulate sensory nerve endings called irritant receptors lying below the airway epithelium.
- Stimulation of these irritant receptors causes parasympathetic nerves to release acetylcholine (ACh). When acetylcholine binds to M3 muscarinic receptors on airway smooth muscle, a series of events is initiated which results in an increase in intracellular calcium and smooth muscle contraction (broncho constriction or bronchospasm).
- Some inflammatory mediators such as histamine can also increase intracellular calcium and cause bronchospasm.
- Inflammation of the airways is brought on the several factors like eosinophil, T-lymphocytes (CD4+), macrophages, and mast cells infiltrate the bronchial wall.
- The epithelium is vacuolated and the ciliated cells desquamate. Several cellular factors play their roles in the inflammatory process.
- Neuropeptides such as bradykinins, substance P and neurotension are lead to broncho constriction and excessive secretion of mucous.
- Mast cells initiate the response on exposure to allergens, excessive osmatic changes and variations in temperature.
- Macrophages produce cytokines, which are either broncho constriction or bronchodilator. Presence of eosinophil in the inflammatory exudate is characteric of asthma.

- Eosinophils are derived from bloodstream. Major basic proteins and cationic proteins of eosinophil lead to destruction of mucosal surface.
- T-lymphocytes, especially CD4+ produce cytokines IL-3,IL-4, IL-5 and GM-CSF which modify the inflammation.
- TNF which is an inflammatory cytokine is expressed in greater amounts by mast cells. The bronco-alveolar secretions contain higher levels of TNF.
- Possibly platelet derived humeral factors also modify the inflammation.
- The main chemical transmitters, which alter the airways, are histamine, prostaglandin and leukotriene. These lead to contraction of bronchial muscle, increase in vascular permeability and excessive secretion of abnormal mucous. Airway inflammation persists for several years. Its severity correlates with the severity of asthma. Hyper responsiveness of the inflamed airways is aggravated by autonomic and neural mechanisms.
- The final result is obstruction of the small and medium sized airways brought about by mucosal oedema, tenacious mucous and broncho constriction. [23]

Classification

Bronchial asthma classified into 2 groups

- Extrinsic asthma (Atopic)
- Intrinsic asthma (Cryptogenic)

Extrinsic

- IgE was raised at least 70%
- Atopic subjects
- Onset was early (10-15 years)
- Intermittent in nature
- Family history of atrophy

Intrinsic

- IgE was normal or low
- Usually Non-atopic subjects
- Onset in middle age (30years)
- Constant in nature
- Family history of asthma.[13]

Clinical Features

Asthma classically displays a diurnal pattern, with symptoms and lung function being worse in early morning.

Typical symptoms are

- Recurrent episodes of wheeze
- Chest tightness
- Breathlessness
- Sometimes Cough

➢ Cough may be a dominant symptom in some asthmatic patients and the lack of wheeze or breathlessness may lead to a delay in reaching the diagnosis of called "cough variant asthma".

➢ The classical aspirin-sensitive patient is female and presents in middle age with asthma, rhino-sinusitis and nasal polyps. Aspirin sensitive patients may also report symptoms following alcohol (white wine) and foods containing salicylates.

Diagnosis

- Diagnosis of bronchial asthma is clinical. The history of sudden attack of paroxysmal dyspnoea, cough and auscultator hallmark of expiratory wheeze heard all over the chest are diagnostic.
- Long duration of complaints history of allergy and positive family history are other helpful clinical points.
- Objective assessment of the severity of airways obstruction and response to broncho dilator therapy can be made by use of bedside peak flow meter.
- Respiratory function tests reveal gross reduction in FEV1, FEV1/FVC ratio and PEFR and increase in the time taken for forced expiration.
- It is important to assess the severity of airways obstruction. Confirmation of the diagnosis of asthma is usually achieved by serial PEFR monitoring. PEFR in the majority of cases shows a diurnal variation of more than 15 % and improvement with therapy.
- When it is necessary to investigate for provocative factors bronchial challenge testing or BPT may be desirable.

Clinical features which indicate severe ventilator impairment are

1. Inability to narrate history continuously or severe distress even on mild exertion
2. Cyanosis, flapping tremors
3. Mental confusion
4. Respiratory rate above 25/min
5. Heart rate persistently above 110/min
6. Inspiratory fall in blood pressure exceeds 16 mm Hg
7. PEFR less than 40 % of predicted value
8. Feeble breath sounds

Differential diagnosis

- Chronic bronchitis
- Cardiac failure
- Pulmonary embolism
- Pulmonary eosinophilia
- Metabolic acidosis
- Emphysema
- Foreign body aspiration.[45]

3. 3. Pharmaceutical review

Vadagam

Definition

Vadagam is similar to pills but larger in size. They are made by grinding with water or juice and then made into vadagam.

Equipment required

1. Mortar and pestle
2. Trays for handling powders and pastes of the drug and for drying the vadagam
3. Vessels and spoons for preparation of juices.

Method of preparation

They preparation of vadagam includes various processes like extraction of juices making decotions, preparing powders grinding paste and rolling into vadagm.

Storage

They should be stored in tightly stoppered glass, tin containers sealed. [46]

Shelf life of medicines

Medicines can be classified into internal and external medicines .They are each in 32 types. Vadagam comes under the category of internal medicines. The shelf life of medicines indicates the potency of medicines. The medicine even though seems to be fresh is not efficacious after sometime. So the medicines should not use after certain period.

As per *Siddha* literature *Agamarunthu padal* in *Gunapadam Thathu-seevam* text,

"உயர்சூர ணம்பிட்டு வடகம் வெண் ணெய்நான்கி

னுயிர்மூன்று திங்களாகும்….."

From the above quote, the shelf life of vadagam is 3 months. [47]

Table1:-Testing parameters for Vadagam -AYUSH guidelines [48]

S.No	Tests
1	Description, Colour, Odour
2	Weight Variation
3	Disintegration Time (Not more than 15 minutes)
4	Identification TLC/ HPTLC/GLC
5	Assay
6	Test for heavy / toxic metals Mercury Arsenic Cadmium Lead
7	Microbial Contamination Total Bacterial count Total Fungal count
8	Test for specific pathogen E.coli Salmonella species Pseudomonas aeruginosa Streptococcus aureus
10	Test for aflatoxins B1, B2, G1, G2

Morden aspect of lozenges

Lozenges are solid preparations that are intended to dissolve or disintegrate slowly in the mouth. They contain one or more medicaments usually in a flavoured, sweetened base. Lozenges are most often used for localized effects in the mouth. They can also be used for systemic effect if the drug is well absorbed through the include phenol, sodium phenolate, benzocaine, and cetylpyridinium chloride. Newer drug include analgesics, anesthetics, antiseptics, antimicrobial, antitussives, anti-nausants, and decongestants.

Lozenges have the advantage of:

1. Being easy to administer to pediatric and geriatric patients.
2. Having formulas that are easy to change and can be patient specific.
3. Keeping the drug in contact with the oral cavity for an extended period of time.

Disadvantage

One disadvantage of using a "gummy- type" lozenge with children is they may perceive it as candy and not a serious dosage form.

Lozenges can be made by molding or by compression. The name troche is applied to compressed lozenges. But in lay language, lozenge and troche are used interchangeably. Commercial lozenges are made by compression, they are harder than ordinary tablets so they will slowly dissolve or disintegrate. Compound lozenges can be prepared by molding mixtures of ingredients containing:

- Sugars to form a hard lozenges
- Polythylene glycol(PEG) to form a soft lozenge
- Gelatin to form a chewable lozenge[49]

3.4. Pharmacological review

Bronchodilator drugs used

- A bronchodilator is a substance that dilates the bronchi and bronchioles, decreasing resistance in the respiratory airway and increasing airflow to the lungs.
- Bronchodilators may be endogenous (originating naturally within the body), or they may be medications administered for the treatment of breathing difficulties.
- They are most useful in obstructive lung disease of which asthma and COPD are the most common conditions.

Types of bronchodilator drugs

Bronchodilators are either short-acting or long acting. Short-acting bronchodilators are used for relief of bronchoconstriction, while long-acting bronchodilators are predominantly used as preventers.

There are three types of bronchodilators namely

1. β2-agonists (short and long-acting)
2. Anticholinergic (short and long- acting)
3. Theophylline (long-acting)

1. β2-agonists

(a) Short-acting β2-agonists

- This medication is providing quick or "rescue" relief from acute bronchoconstriction.
- These medications usually take effect within 20 minutes or less, and can last from 4 to 6 hours.
- These inhaled medications are best for treating sudden and severe or new asthma symptoms.
- Taken 15 to20 minutes ahead of time, these medications can also prevent asthma symptoms triggered by exercise or exposure to cold air.

Examples:

Salbutamol, Levosalbutamol, Pributerol, Terbutaline, Epinephrine, Ephedrine.

(b) Long –acting β2- agonists

- These are long term medications taken routinely in order to control and prevent broncho constriction.
- These medications may take longer to begin working, but relief airway construction for up to 12 hours.
- Commonly taken twice a day with an anti-inflammatory medication, they maintain open airways and prevent asthma symptoms, particularly at night.

  Examples

  Salmeterol, Fenoterol, Pirbuterol, Clebuterol, Formoterol, Bambuterol, Indacaterol[50]

(2) Anticholinergics

- Anti cholinergics or Anti muscarinic drugs relax the smooth muscles but response is slower than Beta-2 agonist.
- Some examples of anticholinergics are Tiotropium bromide and Ipratropium bromide.
- Tiotropium bromide is long acting, a single inhalation can have effect lasting for 24 hours. It reduces the frequency and severity of episodes.
- Ipratropium bromide is short acting, given by inhalation which has effect for 4-6 hours.

(3) Theophylline

- Theophylline is long acting bronchodilator that prevents asthma episodes.
- Available in oral and injectable form.
- It is prescribed in severe cases of Asthma or those that are difficult to control.
- This medication must be taken 1-4 times daily and doses should not be missed.

  Blood tests are required to monitor therapy and to indicate when dosage adjustment is necessary [51]

MECHANISM OF ACTION OF BRONCHODILATOR DRUGS

- Beta-2 agonist drugs bind to beta-2 receptors on airway smooth muscle relaxation. When airway smooth muscle relaxes, the diameter of the air passages is enlarged.

- Bronchodilator drugs blocks the action of phosphordiesterases and prevents the breakdown of cAMP to 5-AMP. This also has the effect to relaxing smooth muscle and allowing the airways to dilate.
- The bronchoconstriction effects of acetylcholine can be blocked by muscarinic antagonists. Muscarinic antagonists bind to muscarinic receptors and prevent acetylcholine from binding.

Bronchodilator can also be achieved by alpha-2 agonist drugs that bind to alpha 2 receptors on parasympathetic nerves and prevent acetylcholine from being released [52]

Pharmacological study in animal models

Bronchodilator activity

In vitro methods

Spasmolytic activity on guinea pigs isolated tracheal chain

The isolated tracheal chain of guinea pigs can be used for testing compounds which inhibit bronchospasm. It detects sympathomimetic, H_1-histamine receptor antagonist properties of test drug.

Methodology

Guinea pig of either sex weighting between 300-500 g are sacrificed using ether anaesthesia. The entire trachea is dissected out and cut into individual rings. Twelve to fifteen rings are tied together with silk threads and mounted in the organ bath containing Krebs-Henseleit solution and maintained at 37^oC, under a tension of 0.5 g and gassed with carbon. Isometric contractions are recovered via a strain gauge transducer on a polygraph. Forty five minutes are allowed for equilibration before the addition of the spasmogen. The following spasmoegns used Histamine, Carbachol, LTC_4 or LTD_4. It takes about 10-12 min for reaching the contraction to a maximum. At this stage, standard and test drugs are administered. The bronchial response is allowed to plateau and recorded. The tissue is rinsed thoroughly and the control contractions are induced again by adding spasmogen. The percent inhibition of spasmogen induced contractions is calculated. From dose response curve ED_{50} is calculated [53]

Isolated Frog Rectus Abdominis Muscle Preparation

A frog is pithed and laid out on frog dissection board. The skin of the anterior abdominal wall is cut by a midline incision which is extended laterally up to the anterior aspects of the limbs. This exposes the flat whitish muscle of the anterior abdominal wall from their pubic origin to their sternal insertion. The two recti are removed and placed in frog ringer solution in a shallow dish. They are carefully cleaned and one of them is trimmed to the desired size and mounted in an organ bath of 5ml capacity, at room temperature, aerated with oxygen. For recording purposes, an isotonic lever with a sideways writing point is used tangential to the smoked drum, balanced for a tension of 2.5gm with an extra load of 1gm on the long arm. A standard solution of Ach is added to the bath and a slow contraction is recorded on the slow moving drum for exactly 90sec. The drum is stopped and the bath fluid is replaced by fresh Frog-Ringer. An extra 1gm load is used to extend the muscle to its original length. [54]

In vivo methods

Histamine induced bronchospasm in guinea pig

Guinea pigs subjected to inhibition of aerosols containing histamine or other bronchospasm inducing agents, exhibits the symptoms of asphyxiating convulsions resembling acute attack of bronchial asthma. These challenging agents are administered in the form of aerosols through a nebulizer to individual guinea pigs placed in a histamine chamber. The initial symptoms are increased frequency of breathing, forced breathing and finally asphyxiating convulsions. The occurrence of these symptoms can be delayed by antagonistic drugs and bronchodilators. Pre-convulsion time is noted as the end point.

Methodology

Male guinea pigs weighing around 400 g are used in groups of 8-10 animals. The animals are treated with test / standard drugs orally or subcutaneously. The animals are then placed in the standard Histamine chamber, 30 min after the administration of drug and exposed to an aerosol of 0.1 % solution of histamine dihydrochloride through a nebulizer. Time required for the onset of asphyxiating convulsions is recorded. The animal is immediately withdrawn from the inhalation box and placed in a well-ventilated area for revival from the convulsions. This method

has been further improvised using an ultra-sound nebulizer which provides the steady exposure to histamine solution at a pre-determined rate. Percent of increase of pre-convulsion time is calculated from the average values of treated and control groups of guinea pigs. ED_{50} values denoting 50% increase in the pre-convulsion time can also be calculated. Histamine aerosol exposure is a very commonly used and dependable method for screening the bronchodilator activity of novel compound.[55]

Egg albumin induced anaphylaxis in guinea pig

Guinea pig was sensitized by two intra peritoneal injections of 0.5 ml and 10% w/v solution of egg albumin at a 48 hours interval. After sensitization, the animals were divided into two groups. Animals of group I received 0.5% CMC and severed as control group. Animals of group II received ethanolic extract trial drug (500 mg/kg. once daily) dissolved in distilled water for 14 days. On day 14, two hours after treatment, the animals were challenged with 0.5 ml of 2% w/v solution of egg albumin into the saphenous vein. Guinea pigs were observed for onset of symptoms such as dyspnoea and cyanosis, duration of persistence of symptoms and mortality [56]

Anti-histamine activity

Effects of diphenhydramine in experimentally produced asthma in guinea pigs

Aim

To demonstrate the antagonistic effects of diphenhydramine against histamine induced bronchospasm in the guinea pig.

Principle

Guinea pig is very sensitive to histamine. When guinea pig is exposed to histamine vapour it exhibits bronchospasm, difficulty in breathing and convulsion. These effects of histamine are mediated through the action of histamine on H_1 receptors. Diphenhydramine is a H_1 receptors blocker. Therefore, diphenhydramine prevents the bronchospasm induced by histamine.

Equipments and other materials required

Histometer, stop watch, disposable needle and syringes.

Animal : Guinea pigs

Drug solutions required

1. Normal saline
2. Diphenhydramine 5 mg/ml
3. Histamine diphosphate 30µg/ml

Procedure

Select 4 guinea pigs having body weight between 250-350 g. Fast the guinea pigs for 12 hours before the experiment. Divide the guinea pigs into 2 groups of 2 animals each. Weigh the guinea pigs in each group and mark them for identification. Administer the drug solutions as Group I Normal saline 1 ml/kg, Group I Diphenhydramine 5 mg/kg. After one hour place each guinea pig in histamine chamber and replace the cover. With the help of compressor, spray a finely atomized mist of histamine diphosphate from nebulizer in both comparments. Using a stop watch record the time of histamine administration. Observe the signs of respiratory distress and the animal falling on its side and record the observations. [57]

Isolated Guinea Pig Ileum

Overnight fasted guinea pigs of either sex weighing 400-600gram were sacrificed using cervical dislocation method. The lower most 10cm of ileum was removed from the abdomen and placed in a shallow dish containing warm Triode solution. Ileum lumen was cleaned by passing through warm 0.9% saline and then segments about one inch in length, were made. The mesenteric attachment and blood etc. were carefully cleaned and tissues was mounted in a thermostatically controlled Dale's organ bath containing 20ml Triode's solution under basal tension of 500mg. the composition of solution in was Nacl, 137; Cacl2, 1.8; Kcl, 2.7; glucose, 5.55; NaHco3, 11.9; Mgcl2, 1; NaH2Po4, 0.4. The solution was continuously bubbled with air. The responses to drug were recorded on a student physiography using isotonic transducer, which exerted a basal tension equivalent to 500mg load tissue. The issue was allowed to equilibrate for 30 min, during which, the bathing solution was changed at every 10 min. Increasing concentration of histamine were added to the bath and the control cumulative concentration-response curve was constructed[58]

Review of siddha drugs

List of Siddha drugs used in Bronchial Asthma

- *Swasakudoori Mathirai*[59]
- *Vasanthakush Magaram*[59a]
- *Gowri Chinthamani*[59b]
- *Thalisadi Chooranam*[8k]
- *Pavala Parpam*[59c]
- *Kashthoori Karuppu*[59d]
- *Adathodai Chooranam*[60]
- *Swasakasa Matthirai*[60a]
- *Kodasoori Kuligai*[59e]
- *Melagu Chooranam*[61]
- *Thalaga karuppu*[59f]
- *Karpooradhi Chooranam*[62]
- *Swasakrudhum*[62a]
- *Sivanar Amirtham* [59g]
- *Kandangkathiri Chooranam*[63]
- *Thooduvalai nei*[59h]
- *Mahathalisapatthira Chooranam*[64]
- *Arakku thailam*[59i]
- *Soombu theneer*[59j]
- *Adhatodai kudineer*[59k]
- *Nochi Thailam*[59l]

3.5. Lateral research

1. Cyperus rotundus

Cyperus have Anti-inflammatory and Anti-arthritic activity. [65]

2. *Zingiber officinale*

Zingiber officinale have anti prolifertive activity, nuroprotective activity, hepato protective activity. [66]

3. C*arum copitum*

Aquaous extract of carum copitum have anxiolytic effect. [67]

4. *Plumbago zeylanica*

The bioactive compound plumbagin and extract of plumbago zeylanic root show a wide spectrum of anti bacterial activity. [68]

5. *Piper nigrum*

Crude extract of black pepper and its main alkaloid, pipperin possess combination of spasmodic activity. [69]

6. *Hyocyamus niger*

Hyocyamus niger have a spasmolytic, antidiarrheal, bronchodialtory and urinary bladder relaxant property. [70]

7. *Terminalia chebula*

Terminalia chebula have anti-oxidant, anti-arthritic, anti-diabetic, cardioproductive and hepatoproductive activity. [71]

8.*Piper longum*

The fruit effectively reduce the passive cutaneous anaphylaxis in rats and protect guinea pigs against antigen induced bronchospasm. [72]

4. MATERIALS AND METHODS

In this dissertation "*Kadukai Vadagam*" was taken as a trial drug from the Siddha literature ''*Athmaratchamirtha Vaidhiya Saara Sangiragam* (part 2)'' of Kandasamy Mudaliyar, published by Dept of Indian Medicine & Homoeopathy 3rd Edition, 2010.

Ingredients of the drug

1. *Kadukkai (Terminalia chebula)* - 500gm
2. *Kalluppu (Sodium chloride)* - 125gm
3. *Korai kizhangu (Cyperus rotandus)* - 12.6 gm
4. *Kurochani omam (Hyoscyamus niger)* - 12.6 gm
5. *Chukku (Zingiber officinale)* - 12.6 gm
6. *Kodiveli Ver (Plumbago indica)* - 12.6 gm
7. *Thippili (Piper longum)* - 12.6 gm
8. *Chevuiyam (Piper nigrum)* - 12.6 gm
9. *Thippili moolam (Piper longum)* - 12.6 gm
10. *Milagu (Piper nigrum)* - 17.5gm
11. *Induppu (Sodium chloride impura)* - 17.5gm
12. *Omam (Carum copticum)* - 17.5gm

Associated drugs

13. Inji charu (*Zingiber officinale*) - 100ml
14. Elumichai charu (*citrus lemon*) - 100ml
15. Butter milk - 100ml

Collection of the raw materials

All the raw materials were bought from Ramasamy chetty country drug store, Parry's corner, Chennai.

Identification and Authentication of the drug

All the raw materials were identified and authenticated by the experts of Gunapadam, Government Siddha medical college, Arumbakkam, Chennai-106. The specimen sample of all the raw drugs have been preserved in PG Gunapadam department individually for future reference. (Reg:GSMC/PG GM/0029-43/2014-2017).

4.1. Preparation of the drug

Purification of the drug

All the raw material were purified individually as per the Siddha literature.

1. Kadukkai- Roasted in pan, inner seed was removed.
2. Kalluppu- It is dissolved in vinegar and clean with a cloth, dried in sun shade.
3. Korai kizhangu –It was sun dried.
4. Kurochani omam-Dust and odd materials were removed.
5. Chukku-It was immersed by using limewater then it was dried in sunlight, finally the above skin was removed.
6. Kodiveli Ver- Soaked in limewater for 15 minutes and taken.
7. Thippili- Immersed in lemon juice and dried.
8. Sevviyam-Outer skin was peeled off and dried in sun.
9. Thippili moolam- Nodes were removed & dried.
10. Milagu-It was immersed in sour buttermilk for 75 minutes and dried.
11. Induppu –Dissolved in vinegar, filtered and dried.
12. Omum –It is immersed in limewater then it was dried in sunlight. [73]

Preparation of the trial drug-Kadukkai Vadagam

After purification all the processed raw material were taken and altogether to obtain fine powder form ginger juice was added to the obtained powder and ground well. This process was repeated with lemon juice and butter milk respectively and made into pills.

Preservation of the drug

Pills were stored in a clean, air tight glass container and labelled as *KV*

Administration of the drug

Route of Administration: Internal

Dose: 1 - 2 Vadagam (2 - 4gm)

Indication: Bronchial asthma, inflammation, pain and dropsy.

4.2. Standardization of the drug

World Health Organization (WHO) has appreciated the importance of medicinal plants for public health care. The process of evaluating the quality and purity of herbo mineral drugs by means of various parameters like physical, chemical and biological

observation is called standardization. Standardization of the this drug comes under the following categories

- Physio-chemical analysis
- Phyto chemical analysis
- Bio chemical analysis

Organoleptic evaluation

The Organoleptic characters of the sample were evaluated which include evaluation of the formulation by its colour, odour, size etc.

1. Colour examination

Ten tablets were taken into watch glasses and positioned against white back ground in white tube light. Its colour was observed by naked eye and results are noted table- 6.

2. Odour examination

Ten numbers of tablets were smelled individually. The time interval among two smelling was kept two minutes to overturn the effect of previous smelling. Odour of *Kadukkai Vadagam* was noted in results table -6.

3. Size examination

The diameter of ten tablets was measured by Vernier caliper. The mean value of diameter was noted.

4.2.1. Physico-chemical investigation

Physico-chemical studies like total ash, water insoluble ash, acid Insoluble ash, loss on drying at 105°C and pH were done at, Central Research Institute, Chennai.

Solubility Test

A pinch of the sample *KV* was taken in a dry test tube and shaken well with distilled water. A little amount of the sample *KV* is shaken well with con.Hcl and then Con. H_2SO4. Solubility was observed.

Determination of Total Ash

About 2 g of the ground drug *KV* was accurately weighed in a silica dish and incinerated at a temperature not exceeding 450º until it was free from carbon, cooled and weighed. The percentage of ash with reference to the air-dried drug was calculated.

Determination of Water Soluble Ash

Total ash was heated up to 600^0C with 25 ml of distilled water for 10 minutes and the residue was ignited in the furnace to get a constant weight. And the weight was calculated.

Determination of Acid Insoluble Ash

The ash obtained was boiled for 5 minutes with 25 ml of dilute hydrochloric acid and insoluble matter was collected in an ash-less filter paper, washed with hot water and put up in flames to constant weight. The percentage of acid-insoluble ash with reference to the air dried drug was noted.

Determination of Moisture Content (Loss on Drying)

This procedure was done to determine the amount of volatile matter in the drug. A sample of 10 gram of the drug *KV* was placed in a tarred evaporating dish after accurately weighting without preliminary drying. The dish was dried at a temperature of 105^0C for about 5 hours and again weighed. The drying and weighing procedure was repeated again and again until the difference between two successive weights was not more than 0.25%. And the weight was calculated.

pH value

Potentiometrically pH value was determined by a glass electrode and a pH meter. The pH of the *KV* was written in results column.

Tablet Disintegration test

Each *KV* was placed in each of the six tubes of the basket present in the disintegration apparatus. The apparatus was operated by using water as the immersion fluid maintained at 35-39 °C. At the end of the 30 min, the basket is lifted from the fluid and the state of the tablet is observed. The disintegration time of *KV* was recorded [74]

Weight variation test

It was carried out to make sure that, each number of tablets contains the proper amount of drug. The test was carried out by weighing the 20 tablets individually using analytical balance, then the average weight was calculated, and comparing the individual tablet weights to the average [75].

The percentage of weight variation is calculated by using this formula.

$$\% \text{ of wt. variation} = \frac{\text{Individual wt.} - \text{Average wt.}}{\text{Average wt.}} \times 100$$

Table 2:- Weight variation limits of Tablets (IP)

Average weight of tablets	Maximum percentage of weight difference allowed
80 mg or less	± 10.0
Between 80 mg and 250 mg	± 7.5
250 mg and more	± 5.0

4.2.2. Phytochemical analysis

The phytochemical screening of *KV* extract was assessed by standard method. Phytochemical screening was carried out on the *KV* extract using aqueous extract to identify the major natural chemical groups such as tannins, saponins, flavonoids, phenols, terpenoids, alkaloids, glycosides, cardiac glycosides, coumarins and steroids. General reactions in these analyses revealed the presence or absence of these compounds in the *Kadukkai Vadagam* extract tested [76]

1. Test for Tannins

For tannin identification, 1 ml of the plant extract, one ml of ferric chloride (5% $FeCl_3$) was added. Formation of dark blue or greenish black indicates the presence of tannins.

2. Test for Saponins

For saponin identification, 2ml Plant extract, 2ml of distilled water was added and shaken in graduated cylinder for 15 minutes lengthwise, and formation of 1cm layer of foam indicates the presence of saponins.

3. Test for Quinones

For Quinones identification, 1ml Plant extract, 1ml of concentrated sulphuric acid (H_2SO_4) was added. Formation of red colour indicates the presence of Quinones.

4. Test for Flavonoids

For flavonoids identification, 2ml of plant extract, 1ml of 2N sodium hydroxide (NaOH) was added. Formation of yellow colour indicates the presence of flavonoids.

5. Test for Alkaloids

For Alkaloids identification, 2ml Plant extract, 2ml of concentrated Hydrochloric acid (Hcl) was added. Then few drops Mayer's reagent was added**.** Presence of green colour or white precipitate indicates the presence of alkaloids.

6. Test for Glycosides

For Glycosides identification, 2ml of the plant extract, 3ml of chloroform and 10% ammonium solution was added. Formation of pink colour indicates the presence of glycosides.

7. Test for Cardiac glycosides

For Cardiac glycosides identification, 0.5 ml of the plant extract, 2 ml of glacial acetic acid and few drops of 5 % ferric chloride were added. This was under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at interface indicates the presence of cardiac glycosides.

8. Test for Terpenoids

For Terpenoids identification, 0.5 ml of the plant extract, 2 ml of chloroform along with concentrated Sulphuric acid. Formation of red brown colour at the interface indicates the presence of Terpenoids.

9. Test for Phenols

For phenol identification, 1ml of the plant extract, 2ml of distilled water followed by few drops of 10 % ferric chloride was added. Formation of blue / green colour indicates the presence of phenols

10. Test for Steroids

For steroid identification, 0.5 ml of the plant extract, 2 ml of chloroform and 1 ml of Sulphuric acid (H_2 SO_4) were added. Formation of reddish brown ring at interface indicates the presence of steroids.

11. Test for Coumarins

For coumarins identification, 1 ml of plant extract, 1 ml of 10 % NaOH was added. Formation of yellow colour indicates the presence of coumarins.

12. Test for Anthocyanin and Beta cyanin

For Anthocyanin and Beta cyanin identification, to 2ml of the plant extract, one ml of 2N sodium hydroxide (NaOH) was added and heated for 5 min at 100 °C. Formation of bluish green colour indicates the presence of anthocyanin and formation of yellow colour indicates the presence of betacyanin.

4.2.3. TLC/ HPTLC finger print studies

HPTLC finger printing was carried out as per the reference [77].

Preparation of spray reagent-vanillin-sulphuric acid reagent

Vanillin (1g) was dissolved in ice cold ethanol (95ml). Add to 5ml of cooled concentrated sulphuric acid. Ice was added and stirred well. The solution was stored in refrigerator.

Chromatographic conditions

Instrument	: CAMAG (Switzerland).
Sample Applicator	: Camag Linomat - IV applicator with N_2 gas flow.
Photo documentation System	: Digi store - 2 documentation system with Win Cat & video scan software.
Scanner	: Camag HPTLC scanner - 3 (030618), Win Cats
IV. Development Chamber	: Camag HPTLC 10X10, 10 X 20 twin trough linear
Development chamber.	
Quantity applied	: 5, 10 µl for extracts and 5 µl for standards
Stationary phase	: Aluminium plate pre-coated with silica gel

60(E. Merck)

Plate thickness : 0.2 mm.

Mobile Phase : For Chloroform extract - Toluene: Ethyl acetate (9:1) and ethanol extract – Toluene: Ethyl acetate (1:1).

Scanning wavelength : 254 nm

Laboratory condition : 26 ± 5°C and 53 % relative humidity

The plate was developed up to a height of 8 cm, air dried, spots were observed under the UV light at 254 and 366 nm. Finally the plates were derivatized using vanillin-sulphuric acid reagent heated at 105° till colour spots appeared.

4.2.4. Bio-chemical analysis

Preliminary Basic and Acidic radical studies

Preparation of extract:

Preparation of the *KV* extracts was assessed by following method. One gram of dried powder of *KV* materials were extracted with 20 mL aqueous for 1 min using an Ultra Turax mixer (13,000 rpm) and soaked overnight at room temperature. The sample was then filtered through Whatman No. 1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a Rota-vator at 40 °C to a constant weight and then dissolved in respective solvents. The dissolving rate of the crude extracts was approximately 100 %. The solution was stored at 18 °C until use [78]

Table.No:3 Test for basic radicals

PROCEDURE	OBSERVATION	INFERENCE
Test for Potassium A pinch of sample is treated with 2ml of sodium nitrate solution and then treated with 2ml of cobalt nitrate in 30% of glacial acetic acid.	Formation of Yellow colour precipitate	Presence of Potassium
Test for Calcium Taken2 ml of extract in a clean test tube.	No Yellow precipitate	Presence of

Then acetic acid and potassium chromate solution were added		Calcium
Test For Magnesium 2ml of extract was taken in a clean test tube, few drops of Magnason reagent was added in drops.	Formation of Blue colour precipitate	Presence of Magnesium
Test For Ammonium 2mlof extract was taken in a test tube and added few ml of Nessler's reagent.	Appearance of Brown colour	Presence of Ammonium
PROCEDURE	**OBSERVATION**	**INFERENCE**
Test For Sodium 2 pinches of *KV* was mixed with HCl and made it into paste. And introduced into the blue flame of Bunsen burner.	Appearance of intense Yellow colour	Presence of Sodium
Test for Iron (Ferrous) 2ml of extract was taken in a clean dried test tube and conc. HNO_3 and ammonium thiocyanate were added.	Appearance of Blood red colour	Presence of Ferrous iron
Test For Zinc 2 ml of the extract was taken in a test tube and Potassium ferro cyanide solution was added.	Formation of White colour precipitate	Presence of Zinc
Test For Aluminium To the 2m1of the extract was taken in a test tube sodium hydroxide drops were added to it.	White precipitate obtained	Presence of Aluminium
Test For Lead 2 ml of extract was taken in a test tube and added with 2ml of potassium iodide solution.	Yellow precipitate obtained	Presence of Lead
Test for Copper		

To a small portion of a extract dil Hcl was added and then hydrogen sulphide gas is passed through the solution.	Black precipitate	Presence of Copper
Test For Mercury 2m1 of the extract was taken in a test tube and treated With 2ml of NaoH solution.	Formation of Yellow precipitate	Presence of Mercury
Test for Arsenic 2m1 of the extract was taken in a test tube and treated with 2ml of NaoH solution.	Brownish red precipitate obtained	Presence of Arsenic

Results were noted and tabulated in Table No:13

Table.No:4 Test for acidic radical

PROCEDURE	OBSERVATION	INFERENCE
Test for Sulphate 2 ml of the extract was taken in clean, dry test tube and 5 % barium chloride solution was added to it.	Formation of white precipitate	Presence of Sulphate
Test for Chloride The extract was taken in a test tube and then treated with Silver nitrate solution.	Formation of White precipitate	Presence of Chloride
Test for Phosphate The extract was taken in a test tube and treated with ammonium molybdate and conc. HNO_3.	Formation of Yellow precipitate	Presence of Phosphate
Test for Carbonate The substance was taken in a clean dry test tube and then treated with Conc. HCl.	Formation of Effervescence	Presence of Carbonate

Test for fluoride & oxalate 2ml of extract was taken in a test tube and added with 2ml of dil.acetic acid, 2ml calcium chloride solution and then heated.	Formation of cloudy appearance	Presence of Fluoride & Oxalate
Test For Nitrate 1gm of the *Kadukkai Vadagam*was heated with copper turnings and concentrated H2SO4 and observed the test tube vertically down.	Characteristic changes	Presence of Nitrate

4.2.5. Availability of microbial load

Enumeration of bacteria by plate count – agar plating technique

The plate count technique was one of the most routinely used procedures because of the enumeration of viable cells by this method.

Principle

This method is based on the principle that when material containing bacteria are cultured, every viable bacterium develops into a visible colony on a nutrient agar medium. The number of colonies therefore is the same as the number of organisms contained in the sample.

Dilution

A small measured volume is mixed with a large volume of sterile water orsaline called the diluent or dilution blank. Dilution is usually made in multiples of ten.

A single dilution was calculated as follows

$$\text{Dilution} = \frac{\text{Volume of the sample}}{\text{Total volume of the sample and the diluents}}$$

Requirements

- Sample or Bacterial suspension

- 9 ml dilution blanks (7)
- Sterile petri dishes (12)
- Sterile 1 ml pipettes(7)
- Nutrient agar medium (200 ml)
- Colony counter.

Procedure

1. Label the dilution blanks as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}.
2. Prepare the initial dilution by adding 1 ml of the sample into a 9 ml dilution blank labeled 10^{-1} thus diluting the original sample 10 times.
3. Mix the contents by rolling the tube back and forth between hands to obtain uniform distribution of organisms.
4. From the first dilution transfer 1 ml of the suspension while in motion, to the dilution blank 10^{-2} with a sterile and fresh 1 ml pipette diluting the original specimen to 100 times.
5. From the 10^{-2} suspension, transfer 1 ml of suspension to 10^{-3} dilution blank with a fresh sterile pipette, thus diluting the original sample to 100 times.
6. Repeat this procedure till the original sample has been diluted 10,000,000 times using every time a fresh sterile pipette.
7. From the appropriate dilutions transfer 1ml of suspension while in motion, with there spective pipettes, to sterile petri dishes. Three petri dishes are to use for each dilution.
8. Add approximately 15 ml of the nutrient medium, melted and cooled to 450c, to each petri dish containing the diluted sample. Mix the contents of each dish by rotating gently to distribute the cells throughout the medium.
9. Allow the plates to solidity.
10. Incubate these plates in an inverted position for 24-48 hours at 370c.

Observation

Observe all the plates for the appearance of bacterial colonies. Count the number of colonies in the plates.

Calculate the number of bacteria per ml of the original suspension as follows:

$$\text{Organisms per millimeter} = \frac{\text{Number of colonies (average of 3 replates)}}{\text{Amount of plated} \times \text{dilution}}$$

Results were noted and tabulated in Table No: 15

4.2.6. Following instrumental analysis is carried out to study quvantitative analysis of *Kadukkai Vadagam*

FT-IR (Fourier Transform Infra-Red)

Model	: Spectrum one: FT-IR Spectrometer
Scan Range	: MIR 450-4000 cm-1
Resolution	: 1.0 cm-1
Sample required	: 50 mg, solid or liquid.

It is the preferred method of infrared spectroscopy. FT-IR is an important and more advanced technique. It is used to identify the functional group, to determine the quality and consistency of the sample material and can determine the amount of compounds present in the sample. It is an excellent tool for quantitative analysis.

In FT-IR infrared is passed from a source through a sample. This infrared is absorbed by the sample according to the chemical properties and some are transmitted. The spectrum that appears denotes the molecular absorption and transmission. It forms the molecular fingerprint of the sample. Like the finger print there is no two unique molecular structures producing the same infrared spectrum. It is recorded as the wavelength and the peaks seen in the spectrum indicates the amount of material present.

FT-IR is the most advanced and the major advantage is its Speed, Sensitivity, Mechanical Simplicity, and Internally Calibrated.

Fig no: 15 Shows Image of FTIR Analyser

XRD (X-RAY POWDER DIFFRACTION)

Definition

X-ray powder diffraction is most widely used for the identification ofunknown crystalline materials (e.g. minerals, inorganic compounds). Determination of unknown solids is important to studies in geology, environmental science, material science and biology.

Applications

- Characterization of crystalline materials[79]

- Identification of fine-grained minerals such as clays and mixed layer clays that are difficult to determine optically
- Determination of unit cell dimensions.

With specialized techniques, XRD can be used to

- Determine crystal structures using Rietveld refinement
- Determine of modal amounts of minerals (quantitative analysis)
- Characterize thin films samples by
- Determining lattice mismatch between film and substrate and toinferring stress and strain
- Determining dislocation density and quality of the film by rockingcurve measurements
- Measuring super lattices in multilayered epitaxial structures
- Determining the thickness, roughness and density of the film usingglancing incidence X-ray reflectivity measurements
- Make textural measurements, such as the orientation of grains, in apolycrystalline sample.

Strengths and Limitations of X-ray Powder Diffraction

Strengths

- Powerful and rapid (< 20 min) technique for identification of an unknownmineral
- In most cases, it provides an unambiguous mineral determination
- Minimal sample preparation is required
- XRD units are widely available
- Data interpretation is relatively straight forward.

Fig no:16 Shows Image XRD Analyser

Limitations

- Homogeneous and single phase material is best for identification of unknown
- Must have access to a standard reference file of inorganic compounds
- Requires tenths of a gram of material which must be ground into a powder
- For mixed materials, detection limit is ~ 2% of sample
- For unit cell determinations, indexing of patterns for non-isometric crystalsystems is complicated.

Sample Collection and Preparation

Determination of an unknown requires: the material, an instrument for grinding, and a sample holder.

- Obtain a few tenths of a gram (or more) of the material, as pure as possible
- Grind the sample to a fine powder, typically in a fluid to minimize inducing extra strain (surface energy) that can offset peak positions, and to randomizeorientation.

- Powder less than ~10 μm(or 200-mesh) in size is preferred place into a sample holder or onto the sample surface.

ICP-OES (INDUCTIVELY COUPLED PLASMA OPTIC EMISSION SPECTROMETRY)

Fig no: 17 Shows Image of ICP-OES Analyser

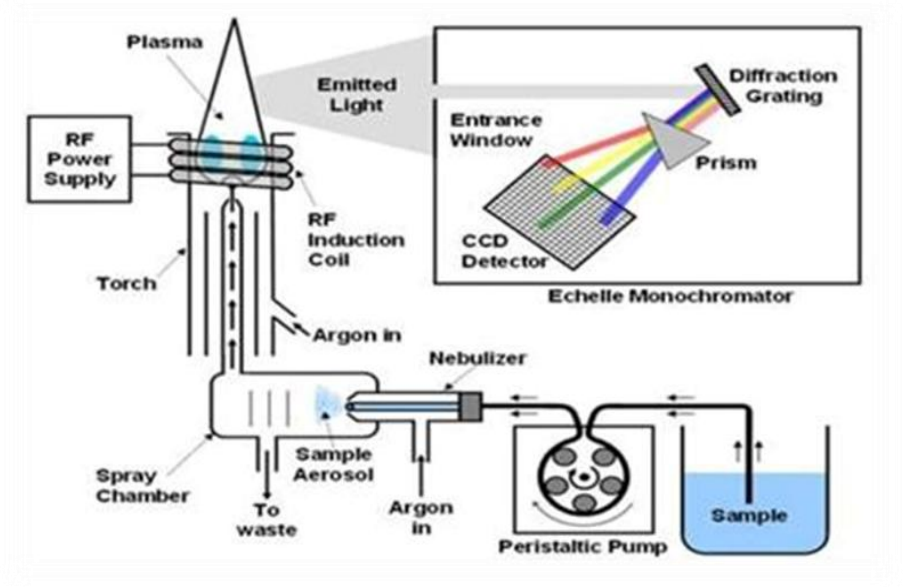


Fig no: 17 Shows Image of ICP-OES Analyser

Manufacturer: Perkin Elmer Model

Optima 5300 DV ICP-OES Inductively Coupled Plasma Spectrometer (ICP) Principle: An aqueous sample is converted to aerosols via a nebulizer. The aerosols are transported to the inductively coupled plasma which is a high temperature zone (8,000–10,000ºC). The analysts are heated (excited) in different (atomic and/or ionic) states and produce characteristic optical emissions (lights). These releases are separated based on their respective wavelengths and their strengths are measured (spectrometry). The intensities are proportional to the concentrations of analyses in the aqueous sample. The quantification is an external multipoint linear standardization by comparing the emission intensity of an unknown sample with that of a standard sample. Multi-element calibration standard solutions are prepared from single- and multi element primary standard solutions. With respect to other kinds of analysis where chemical speciation is relevant (such as the concentration of ferrous iron or Ferric Iron), only total essential concentration is analysed by ICP-OES [80]

Application

The analysis of major and minor elements in solution samples.

Objectives

- Determine elemental concentrations of different metals.
- Learn principles and operation of the ICP-OES instrument
- Develop and put on a method for the ICP-OES sample analysis
- Enhance the instrumental conditions for the analysis of different elements Probes the outer electronic structure of atoms.

Mechanism

In plasma emission spectroscopy (OES), a sample solution is presented into the core of Inductively coupled argon plasma (ICP), which generates temperature of approximately 8000°C. At this temperature all elements become thermally excited and emit light at their characteristic wavelengths. This light is collected by the spectrometer and passes through a diffraction grating that serves to resolve the light into a spectrum of its essential wavelengths. Within the spectrometer, this deflected light is then collected by wavelength and amplified to yield an strength of measurement that can be converted to an elemental concentration by comparison with standardization values. The Inductively coupled plasma optical emission spectrometric (ICP-OES) analysis was done in SAIF, IIT MADRAS, Chennai-36 using Perkin Elmer Optima 5300 DV. Sample preparation: Inductively Coupled Plasma Spectroscopy techniques are the so-called "wet" sampling methods whereby samples are introduced in liquid form for analysis. 100 mg RSP was occupied in a clean, dry test tube. To this, 3 ml Nitric acid was added and mixed well and allowed for few minutes until the reactions were completed. And then, 25 ml of Refined water, was added to prepare digested solution.

The digested sample solution was shifted into plastic containers and labelled properly. It was completed in Bio-chemistry lab, Govt. Siddha Medical College, Chennai-106.

SEM (Scanning Electron Microscope)

In scanning electron microscope high-energy electron beam is focused through a probe towards the sample material. Variety of signals was produced on interaction with

the surface of the sample. This results in the emission of electrons or photons and it is collected by an appropriate detector.

The types of signal produced by a scanning electron microscope include

- Secondary electrons
- back scattered electrons
- characteristic x-rays, light
- specimen current
- Transmitted electrons.

This gives the information about the sample and it includes external morphology, texture, its crystalline structure, chemical composition and it displays the shape of the sample.

Fig: 18 Shows Image SEM Analyser

4.3. Acute oral toxicity study of Kadukkai Vadagam (OECD guideline – 423) [81]

Introduction

- The acute toxic class method is a stepwise procedure with the use of 3 animals of a single sex per step.
- Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgement on the acute toxicity of the test substance.
- This procedure is reproducible, uses very few animals and is able to rank substances in a similar manner to the other acute toxicity testing methods.

- The acute toxic class method is based on biometric evaluations with fixed doses, adequately separated to enable a substance to be ranked for classification purposes and hazard assessment.
- In principle, the method is not intended to allow the calculation of a precise LD50, but does allow for the determination of defined exposure ranges where lethality is expected since death of a proportion of the animals is still the major endpoint of this test.
- The method allows for the determination of an LD50 value only when at least two doses result in mortality higher than 0% and lower than 100%.
- The use of a selection of pre-defined doses, regardless of test substance, with classification explicitly tied to number of animals observed in different states improves the opportunity for laboratory to laboratory reporting consistency and repeatability.

Principle of the Test

It is the principle of the test that based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.

− no further testing is needed

− dosing of three additional animals, with the same dose

− dosing of three additional animals at the next higher or the next lower dose level. The method will enable a judgment with respect to classifying the test substance to one of a series of toxicity classes.

Methodology

Selection of Animal Species

The preferred rodent species is the wistar albino rat, although other rodent species

may be used. Healthy young adult animals are commonly used laboratory strains should be employed. Females should be nulliparous and non-pregnant. Each animal, at the commencement of its dosing, should be between 6 to 8 weeks old and the weight (150-200gm) should fall in an interval within±20 % of the mean weight of any previously dosed animals.

Housing and Feeding Conditions

The temperature in the experimental animal room should be 22ºC ± 3ºC. Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be group-caged by dose, but the number of animals per cage must not interfere with clear observations of each animal.

Preparation of animals

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions

Test Animals and Test Conditions

Sexually mature Female Wistar albino rats (150-200gm) were obtained from Kings institute, Chennai. All the animals were kept under standard environmental condition (22±3°C). The animals had free access to water and standard pellet diet (Sai meera foods, Bangalore).

Preparation of animals

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions

Preparation for acute toxicity studies

Rats were deprived of food overnight (but not water 16-18 h) prior to administration of the, Kadukkai Vadagam

The principles of laboratory animal care were followed and the Institutional Animal Ethical Committee approved the use of the animals and the study design

IAEC approved Number: IAEC/XLVIII/07/CLBMCP/2016

Test Substance	: *Kadukkai Vadagam*
Animal Source	: Kings Institute, Chennai.
Animals	: Wister Albino Rats (Female-3+3)
Age	: >6 weeks
Body Weight on Day 0	:180-280 gm.
Acclimatization	: Seven days prior to dosing.
Veterinary examination	: Prior and at the end of the acclimatization period.
Identification of animals	: By cage number, animal number and individual marking by using Picric acid.
Number of animals	: 3 Female/group,
Route of administration	: Oral
Diet	: Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore.
Water	: Aqua guard portable water in polypropylene bottles.
Housing & Environment	: The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	: Between 22ºC + 3ºC.
Relative humidity	: Between 30% and 70%,
Air changes	: 10 to 15 per hour and
Dark and light cycle	: 12:12 hours.
Duration of the study	: 14 Days

Administration of Doses

Kadukkai Vadagam was suspended in water and administered to the groups of wistar albino rats in a single oral dose by gavage using a feeding needle. The control group received an equal volume of the vehicle. Animals were fasted 12 hours prior to dosing. Following the period of fasting, the animals were weighed and then the test

substance was administered. Three Female animals are used for each group. The dose level of 3, 30,300 and 2000 mg/kg body weight was administered stepwise. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously as per the guideline after substance administration. The visual observations included skin changes, mobility, aggressively, sensitivity to sound and pain, as well as respiratory movements.Finally, the number of survivors was noted after 24 hrs and these animals were then monitored for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

Observations

Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed. All observations are systematically recorded with individual records being maintained for each animal.

Observations include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. The principles and criteria summarized in the Humane Endpoints Guidance Document taken into consideration. Animals found in a moribund condition and animals showing severe pain or enduring signs of severe distress was humanly killed. When animals are killed for human reasons or found dead, the time of death was recorded.

Behaviour

The animals will be observed closely for behaviour in the first four hours which includes abnormal gait, aggressiveness, exophthalmos, ptosis, akinesia, catalepsy,

convulsion, excitation, head twitches, lacrimation, loss of corneal reflex, loss of traction, piloerection reactivity of touch, salivation, scratching, sedation, chewing, head movements, sniffing, straub, tremor and writhes, diarrhea, leathery, sleep and coma.

Body Weight

Individual weight of animals was determined before the test substance was administered and weights will be recorded at day 1, 7, and 14 of the study. Weight changes were calculated and recorded. At the end of the test, surviving animals were weighed and humanly killed.

Food and water Consumption

Food and water consumed per animal was calculated for control and the treated dose groups.

Mortality

Animals were observed for mortality throughout the entire period.

REPEATED DOSE 28-DAY ORAL TOXICITY STUDY OF *KADUKKAI VADAGAM* ON RATS-(OECD 407 guidelines) [82]

Test Substance	: *KADUKKAI VADAGAM*
Animal Source	: TANUVAS,Madhavaram, Chennai.
Animals	: Wister Albino Rats (Male -24, and Female-24)
Age	: >6 weeks
Body Weight	: 160-180 gm.
Acclimatization	: Seven days prior to dose.
Veterinary examination	: Prior and at the end of the acclimatization period.
Identification of animals	: Bycage number, animal number and individual marking by using Picric acid
Diet	: Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore.
Water	: Aqua guard portable water in polypropylene bottles.
Housing & Environment	: The animals were housed in Polypropylene cages provided with bedding of husk.

Housing temperature	: Between 22ºC ± 3ºC.
Relative humidity	: Between 30% and 70%,
Air changes	: 10 to 15 per hour
Dark and light cycle	: 12:12 hours.
Duration of the study	: 28 Days.

Table 5

Groups	No of Rats
Group I Vehicle control (Normal Saline)	12 (6 male, 6 female)
Group II Kadukkai Vadagam 30 mg / kg	12 (6 male, 6 female)
Group III Kadukkai Vadagam 150 mg / kg	12 (6 male, 6 female)
Group IV Kadukkai Vadagam 300 mg / kg	12 (6 male, 6 female)

Methodology

Randomization, Numbering and Grouping of Animals:

48 Wistar Albino Rats (24M + 24F) were selected and divided into 4 groups. Each group consist of 12 animals (Male -6, and Female-6). First group treated as a control and other three group were treated with test drug (low, mid, high) for 28 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was marked with picric acid. The females were nulliparous and non-pregnant.

Justification for Dose Selection

As per OECD guideline three dose levels were selected for the study. They are low dose(30 mg/kg), mid dose dose (150 mg/kg), high dose (300 mg/kg). X is calculated by multiplying the therapeutic dose of human (2000mg/kg) and the body surface area of the rat (0.018). i.e X dose is 30 mg/kg/animal ,5Xmid dose is 150 mg/kg, 10X high dose is 300 mg/kg.

Preparation and Administration of Dose

Kadukkai Vadagamsuspended in with water, It was administered to animals at the dose levels of 30, 150 and 300 mg/kg. The test substance suspensions were freshly prepared every two days once for 28 days. The control animals were administered vehicle only. The drug was administered orally by using oral gavage oncedaily for 28 consecutive days.

Observations

Experimental animals were kept under observation throughout the course of study for the following

Body Weight

Weight of each rat was recorded on day 0, at weekly intervals throughout the course of study.

Food and water Consumption

Food and water consumed per animal was calculated for control and the treated dose groups.

Clinical signs

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

Mortality

All animals were observed twice daily for mortality during entire course of study.

Necropsy

All the animals were sacrificed by excessive anaesthesia on day 29. Necropsy of all animals was carried out.

Laboratory Investigations

Following laboratory investigations were carried out on day 29 in animals fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Bio chemistry and potassium EDTA (1.5 mg/ml) for Hematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes.

Haematological Investigations

Haematological parameters were determined using Haematologyanalyzer.

Biochemical Investigations

Biochemical parameters were determined using auto-analyzer.

Histopathology

Control and highest dose group animals will be initially subjected to histopathological investigations. If any abnormality found in the highest dose group than the low, then the mid dose group will also be examined. Organs will be collected from all animals and preserved in 10% buffered neutral formalin for 24 h and washed in running water for 24 h. The organ sliced 5 or 6µm sections and were dehydrated in an auto technicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained withHaematoxylin-eosin red .[83]

Statistical analysis

Findings such as body weight changes, water and food consumption,hematology and blood chemistry were subjected to One-way ANOVA followed by dunnett test using a computer software programme – Graph pad version 5.0 .All data were summarized in tabular form, (Table)

4.4. Pharmacological activity

4.4.1. In Vivo Bronchodilator activity of *Kadukkai Vadagam* Guinea pig

Histamine induced bronchoconstriction in guinea pig

Overnight fasted guinea pigs were divided into six groups each containing 6 animals.

- Group 1 was treated as control,
- Group 2 received standard drug chlorpheniramine maleate (2 mg / kg).
- Groups 3 *Kadukkai Vadagam* 200 mg / kg.

- Group 4 *Kadukkai Vadagam* 400mg / kg

All the doses were given orally once a day for 5 days. Prior to drug treatment each animal was placed in the histamine chamber and exposed to 0.2 % histamine aerosol. The pre convulsive time (PCT) was determined from the time of exposure to onset of convulsions. As soon as the PCT were noted, the animal were removed from the chamber and placed in fresh air. Group 2 received Chlorpheniramine maleate. These animals were again subjected to histamine aerosol after 1hr.of drug administration and PCT was determined. The protection offered by treatment was calculated by using the formula[1, 2].

Percentage Protection = (1 - T1/T2) X 100 Where,

T1 = the mean of PCT before administration of test drugs.

T2 = the mean of PCT after administration of test drugs.

4.4.2. In vitro antihistaminic activity of *Kadukkai Vadagam* isolated guinea pig ileum

Experimental Procedure:

Guinea pig was sacrificed and a segment from ileum (2 cm) was dissected from the terminal ileum and mounted in an organ bath containing Tyrode solution (10 ml) between two stainless steel hooks under 0.5 to 1 g initial tension. The lower hook was fixed at the bottom of the organ bath and upper one was connected to an isotonic transducer. The Tyrode solution composition (pH 7.4) was NaCl-8.0, KCl-0.2, $CaCl_2$-0.2, $MgCl_2$-0.1, $NaHCO_3$ .1.0, NaH_2PO_4-0.05, and Glucose-10.0gms/liter.

It was continuously aerated and maintained at 37 ± 0.5ºC. The equilibrium period was 60 min and the bath solution was refreshed every 15 min. After equilibrium period, a dose response curve for histamine in variant molar concentrations, by maintaining 45 min time cycle was taken separately. Results are provided in the table

Statistical Analysis

Ileum contractions induced by agonist were assumed as 100% and reductions induced by test drug calculated. Percentage of ileum contraction was expressed as mean ±

SEM. Results were analyzed using one-way analysis of variance (ANOVA). Probability value less than 0.05 were considered as significant. Results are discussed in table.

4.4.3. Anti-oxidant activity oF *Kadukkai Vadagam*

DPPH ASSAY (2, 2-diphenyl -1-picrylhydrazyl) in In-Vitro

The radical scavenging activity of different extracts was determined by using DPPH assay according to[84].The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517 nm.

Ascorbic acid (10mg/ml DMSO) was used as reference.

Principle

1,1-diphenyl-2-picryl hydrazyl is a stable free radical with red colour which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as,

$$DPPH + [H\text{-}A] \rightarrow DPPH\text{-}H + (A)$$

Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

Reagent Preparation

0.1 mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol.

Procedure

Different volumes (1.25-20µg/µl) of *KV* extracts were made upto 40µl with DMSO and 2.96ml DPPH (0.1mM) solution was added. There action mixture was incubated in dark condition at room temperature for 20min.After20min,the absorbance of the mixture was read at 517 nm. 3ml of DPPH was taken as control. The % radical scavenging activity of the *KV* extracts was calculated using the following formula,

$$\% \text{ inhibition} = \frac{\text{Control - Test}}{\text{Control}} \times 100$$

Qualitative analysis of antioxidant activity of *KV* extract

The antioxidant activity of *KV* extract was determined by following the method [85].

Quantitative analysis of free radical scavenging activity of *KV* extract

The antioxidant activities were determined using DPPH (Sigma-Aldrich) as a free radical. 100µl of *KV* extract were mixed with 2.7ml of methanol and then 200µl of 0.1 % methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control (Lee *et al.*, 2005). Subsequently, at every 5 min interval, the absorption maxima of the solutions were measured using a UV double beam spectra scan (Chemito, India) at 517nm. The antioxidant activity of the sample was compared with known synthetic standard of 0.16% Butylated Hydroxy Toluene (BHT). The experiment was carried out in triplicates. Free radical scavenging activity was calculated by the following formula:

% DPPH radical-scavenging = [(Absorbance of control - Absorbance of test Sample) / (Absorbance of control)] x 100.

5. RESULTS AND DISCUSSION

Standardization of the test drug

Standardisation of the drug is more essential to derive the efficacy, potency of the drug by analysing it by various studies. Following are the results of physio chemical and phytochemical analysis. Physical characterisation and estimation of basic and acidic radicals have been done and tabulated.

Toxicological results of the drug and pharmacological activity of the drug are derived. Its result has been tabulated and interpretation is made below. Thus, it is to give a complete justification, to bring the effectiveness of the trial drug *Kadukkai Vadagam.*

Results

Table: 6 Organoleptic characterization of *Kadukkai Vadagam*

S.No	Parameter		Results
1.	Colour		Dark Brown
2.	Odour		Citrus smell
3.	Taste		Sour in taste
4.	Sense of touch		Hard
5.	Size		1 cm
6.	Solubility		
	i	Distilled water	Soluble
	ii	Benzene	Soluble
	iii	Chloroform	Soluble
	iv	Carbon tetra chloride	Soluble
	v	Xylene	Soluble
	vi	Petroleum ether	Soluble
	vii	Propylene glycol	Not Soluble

Table: 7 Physicochemical Analysis

S.No	Parameter	Result
1.	PH	3.93
2.	Total Ash	15.12%
3.	Acid Insoluble ash	0.68%
4.	Water soluble ash	3.13%
5.	Loss on drying at 105^0C	14.22%
6.	Water soluble Extractive	44.00%
7.	Alcohol soluble Extractive	45.81%
8.	Disintegration time	24 min

Discussion

pH value

- The pH of *KV* is 3.93 It is weak acidic in nature.
- This pH level plays a role in enzyme activity by maintaining the chemical environment thus regulating the homeostasis.
- It is also an important factor for drug absorption. Being weak acidic, the drug is more readily absorbed in an acid medium like stomach which enhance the bio availability of the drug.

Total ash

- Total ash value will determine the amount of minerals and earthy materials present in the drug.
- The total ash value of *KV* is 15.12% which determines the presence of inorganic content.

Acid insoluble ash

- The acid insoluble ash value of the drug denotes the amount of siliceous matter (dust, sand etc.,) present in that drug.
- The quality of the drug is better if the acid insoluble ash value is low.
- Here, acid insoluble ash value of *KV* is 0.68%. Hence, it represents the superior quality of the *KV*.

Water soluble ash

- Water soluble ash is a part of total ash value, which denotes the colloidal or crystalline nature of the drug.

- Here, the water soluble ash value of is 3.13%, which represents easy facilitation of diffusion and osmosis mechanism.

Loss on Drying (LOD)

- It indicates the amount of volatile substance and moisture present in the drug.
- This also indicates the stability and shelf life of the drug.
- The loss on drying percentage of *KV* is 14.22

Being a Vadagam, without incineration process, the moisture content is slightly high.

Weight variation test

Table : 8 Uniformity weight variation test result of *KV*

S.No.	Weight of each *Vadagam (g)*	% of weight variation	Maximum weight variation with in ± 5%
1	1.9	- 4.0	Yes
2	1.9	- 4.0	Yes
3	2.0	+ 1.0	Yes
4	2.0	+ 1.0	Yes
5	2.0	+ 1.0	Yes
6	2.0	+ 1.0	Yes
7	1.9	- 4.0	Yes
8	2.0	- 4.0	Yes
9	18	- 9.0	No
10	2.0	+ 1.0	Yes
11	2.0	+ 1.0	Yes
12	2.0	+ 1.0	Yes
13	2.2	+ 11	No
14	2.0	+ 1.0	Yes
15	2.1	+ 6.0	No
16	2.0	+ 1.0	Yes
17	2.0	+ 1.0	Yes
18	2.0	+ 1.0	Yes
19	1.9	- 4.0	Yes
20	1.9	- 4.0	Yes
			Average: 1.98 g

Discussion

- Average weight of the *KV* was noted as 1.98g. Out of 20 tablets tested, 17 tablet of them lies within ± 5% weight variation and 3 tablet above the limit.
- According to the limits of weight test cited in the Indian pharmacopoeia, *KV* passed the Uniformity weight test.
- The uniformity test resembles uniformly distribution of this tablet helps good absorption and distribution.

Table: 9 Results of Phytochemical screening test

Phytochemicals Tested	***KV* Aqueous extract**
Alkaloids	Absent
Glycosides	Present
Saponin	Present
Carbohydrate	Present
Phytosterol	Present
Phenol	Present
Triterpene	Present
Flavonoid	Absent
Quinone	Absent
Protein	Present

Discussion

Phytochemicals are natural bioactive compounds, found in plants and fibres, which act as a defence system against diseases and more accurately to protect against diseases**.** The phytochemical analysis reveals the presence of Glycosides, Saponin, Carbohydrate, Phytosterol, Phenol, Triterpenes and Protein.

Glycosides

- Many plants store chemicals in the form of inactive glycosides, such plant glycosides are used as medications.
- Glycosides inhibit eosinophil accumulation in tissue and allergic inflammation.

- They can be effectively used for preventing or treating allergic diseases associated with inflammation and eosinophil accumulation such as COPD, Bronchial asthma and Allergic rhinitis [85]

Carbohydrates

- Carbohydrates provide energy for physical activity and functions of the body.
- Repair of epithelial tissue injury in asthma was made by carbohydrates [86].

Phytosterol

- Plants steroids have potential anti-inflammatory effect. They are important to cure the chronic inflammatory diseases like bronchial asthma [87].
- Phytosterol exerts anti-oxidant effect [88].

Saponins

- It has anti-spasmodic, anti-inflammatory, expectorant and anti-oxidant property.
- Saponins quicker the expulsion of mucus from the lungs [89].

Phenols

- Phenol groups are the essential part of many anti-oxidant compounds.
- They possess rich Anti-Oxidant property and protect body from oxidative stress.
- It has anti-inflammatory property [90].

Triterpene

- They possess Anti-oxidant, Anti-inflammatory and mucolytic activity.
- Suppress the inflammatory response
- They are often expectorant

Proteins

- Protein is an important component of every cell in the body.
- Body uses protein to build and repair tissues.
- Amino acids delay the progressive nature of the diseases and aging process.

A synergistic effect of all these Glycosides, Saponin, Carbohydrate, Phytosterol, Phenol, Triterpenes and Protein increases the potency of the drug against Bronchial asthma. [92]

TLC/HPTLC analysis of chloroform extract

HPTLC analysis

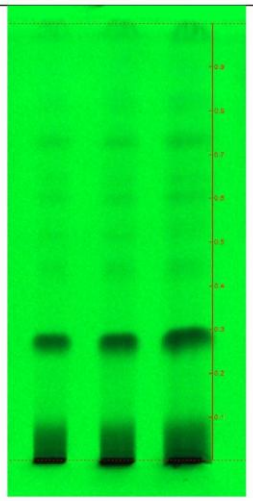
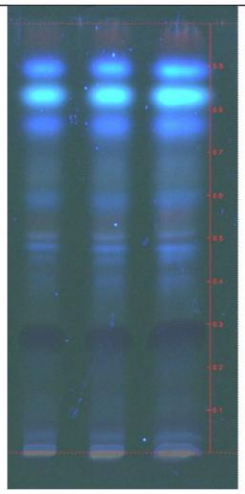
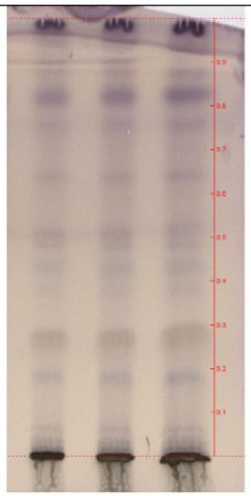
Chloroform extract was applied in TLC aluminium sheet silica gel 60 (E. MERCK) and plate was developed using the solvent system Toluene: Ethyl acetate (9:1). After development, the plate is allowed to dry in air and examined under UV - 254nm, 366 nm and Visible light (Vanillin - Sulphuric acid).

TLC Photo documentation : *Kadukkai Vadagam*

Stationary Phase - Silica Gel 60 F_{254}

Mobile Phase – Toluene:ethyl acetate:formic acid (1:1:0.2)

Table: 10 Rf Values for the chloroform extract of *KV*

5µl 7µl 10µl		5µl 7µl 10µl		5µl 7µl 10µl	
λ =254 nm		**λ =366 nm**		**λ =520 nm**	
R_f	**Colour**	**R_f**	**Colour**	**R_f**	**Colour**
0.28	Green	0.04	Blue	0.06	Grey
0.44	Green	0.27	Dark blue	0.18	Grey
0.52	Green	0.48	Blue	0.27	Brown
0.59	Green	0.51	Blue	0.44	Grey
0.73	Green	0.60	Blue	0.49	Grey
0.82	Green	0.77	Bright blue	0.51	Grey
		0.85	Bright blue	0.64	Grey
		0.90	Bright blue	0.76	Grey
				0.82	Grey

HPTLC Chromatogram of scanning

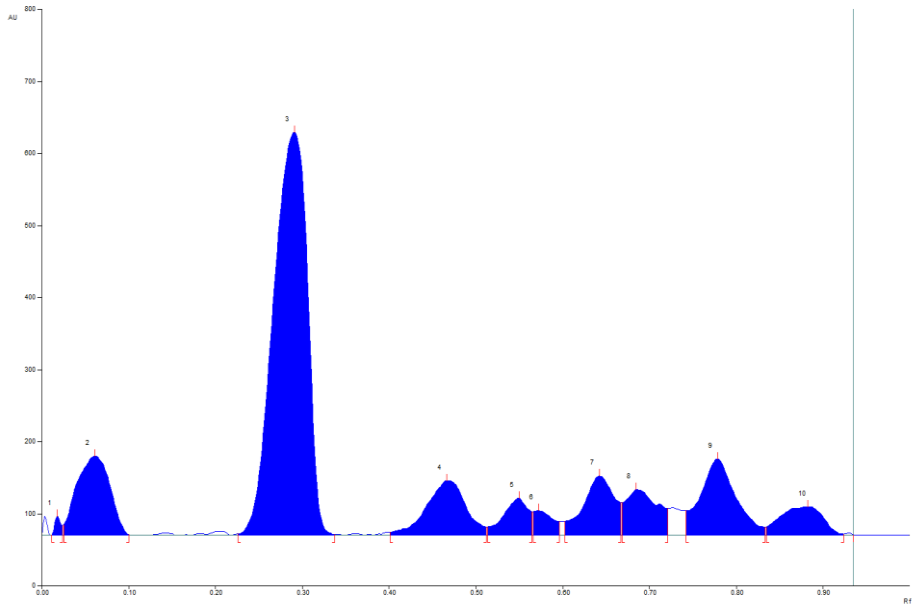


Fig no:19 shows HPTLC Chromatogram of scanning at 254 nm - 5µl

Table no: 11 Chromatogram of scanning at 254 nm - 5µl

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.0 AU	0.02 Rf	26.4 AU	2.31 %	0.02 Rf	13.0 AU	170.0 AU	0.42 %
2	0.03 Rf	13.9 AU	0.06 Rf	109.7 AU	9.58 %	0.10 Rf	0.5 AU	3881.6 AU	9.66 %
3	0.23 Rf	1.9 AU	0.29 Rf	559.0 AU	48.80 %	0.34 Rf	0.8 AU	20294.9 AU	50.49 %
4	0.40 Rf	4.0 AU	0.47 Rf	75.3 AU	6.58 %	0.51 Rf	11.4 AU	3160.7 AU	7.86 %
5	0.51 Rf	11.4 AU	0.55 Rf	51.2 AU	4.47 %	0.56 Rf	32.5 AU	1354.5 AU	3.37 %
6	0.57 Rf	32.6 AU	0.57 Rf	33.7 AU	2.94 %	0.60 Rf	18.9 AU	693.2 AU	1.72 %
7	0.60 Rf	19.5 AU	0.64 Rf	82.0 AU	7.15 %	0.67 Rf	45.0 AU	2696.1 AU	6.71 %
8	0.67 Rf	45.1 AU	0.69 Rf	62.9 AU	5.49 %	0.72 Rf	37.1 AU	2159.4 AU	5.37 %
9	0.74 Rf	33.7 AU	0.78 Rf	105.7 AU	9.22 %	0.83 Rf	10.8 AU	3978.4 AU	9.90 %
10	0.83 Rf	11.1 AU	0.88 Rf	39.6 AU	3.46 %	0.92 Rf	1.5 AU	1803.3 AU	4.49 %

HPTLC Chromatogram of scanning

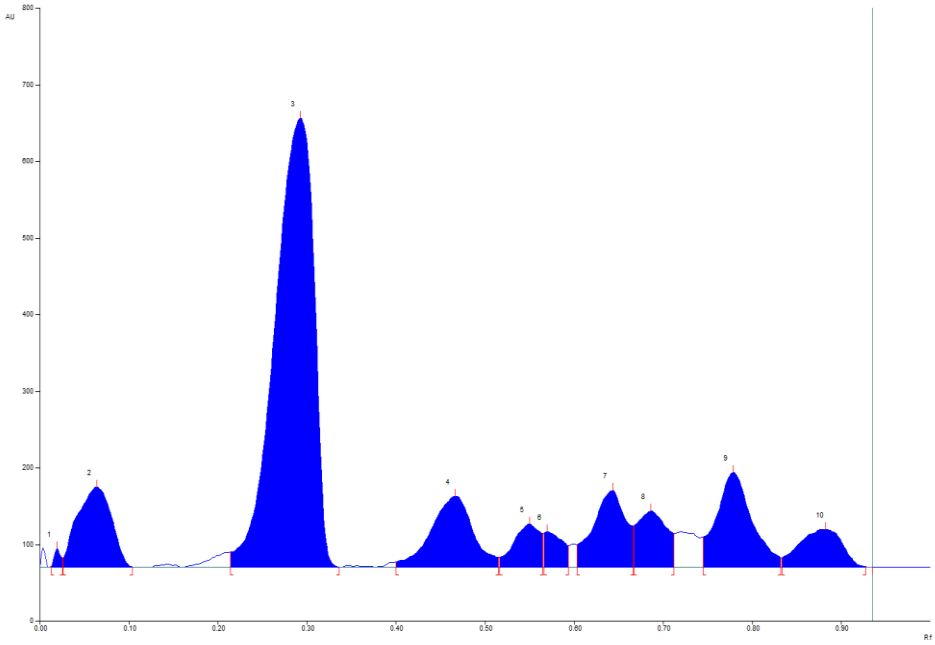


Fig no: 20 shows Chromatogram of scanning at 254 nm - 7µl

Table no: 12 Chromatogram of scanning at 254 nm - 7µl

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.5 AU	0.02 Rf	23.8 AU	1.90 %	0.03 Rf	11.6 AU	157.0 AU	0.34 %
2	0.03 Rf	12.2 AU	0.06 Rf	104.5 AU	8.32 %	0.10 Rf	0.0 AU	3731.5 AU	8.01 %
3	0.21 Rf	19.6 AU	0.29 Rf	586.1 AU	46.65 %	0.34 Rf	0.0 AU	23848.1 AU	51.20 %
4	0.40 Rf	6.9 AU	0.47 Rf	92.9 AU	7.39 %	0.51 Rf	12.7 AU	4023.4 AU	8.64 %
5	0.52 Rf	12.9 AU	0.55 Rf	56.3 AU	4.48 %	0.56 Rf	44.1 AU	1544.8 AU	3.32 %
6	0.57 Rf	44.6 AU	0.57 Rf	46.4 AU	3.69 %	0.59 Rf	27.7 AU	897.8 AU	1.93 %
7	0.60 Rf	29.4 AU	0.64 Rf	100.2 AU	7.98 %	0.67 Rf	53.7 AU	3360.3 AU	7.21 %
8	0.67 Rf	54.0 AU	0.69 Rf	73.0 AU	5.81 %	0.71 Rf	44.3 AU	2244.1 AU	4.82 %
9	0.75 Rf	39.5 AU	0.78 Rf	123.5 AU	9.83 %	0.83 Rf	12.4 AU	4556.0 AU	9.78 %
10	0.83 Rf	12.4 AU	0.88 Rf	49.4 AU	3.94 %	0.93 Rf	0.7 AU	2219.1 AU	4.76 %

HPTLC Chromatogram of scanning

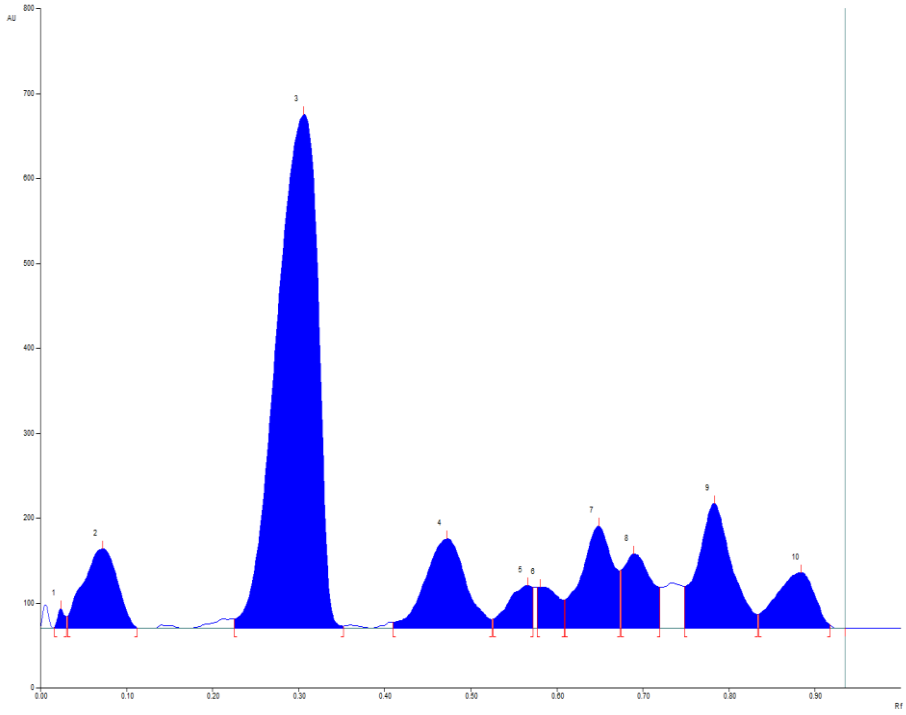


Fig no: 21 shows Chromatogram of scanning at 254 nm-10µl

Table no: 13 Chromatogram of scanning at 254 nm - 10 µl

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	1.4 AU	0.02 Rf	22.7 AU	1.69 %	0.03 Rf	13.4 AU	174.5 AU	0.33 %
2	0.03 Rf	14.0 AU	0.07 Rf	93.4 AU	6.94 %	0.11 Rf	0.1 AU	3443.5 AU	6.60 %
3	0.22 Rf	11.1 AU	0.31 Rf	605.3 AU	44.97 %	0.35 Rf	2.3 AU	26831.1 AU	51.39 %
4	0.41 Rf	6.9 AU	0.47 Rf	105.2 AU	7.82 %	0.52 Rf	10.4 AU	4601.4 AU	8.81 %
5	0.53 Rf	10.6 AU	0.57 Rf	50.6 AU	3.76 %	0.57 Rf	48.3 AU	1310.2 AU	2.51 %
6	0.58 Rf	48.4 AU	0.58 Rf	48.7 AU	3.62 %	0.61 Rf	33.2 AU	1109.9 AU	2.13 %
7	0.61 Rf	33.6 AU	0.65 Rf	120.3 AU	8.94 %	0.67 Rf	67.5 AU	4093.8 AU	7.84 %
8	0.68 Rf	67.9 AU	0.69 Rf	87.5 AU	6.50 %	0.72 Rf	48.4 AU	2615.8 AU	5.01 %
9	0.75 Rf	49.2 AU	0.78 Rf	146.5 AU	10.88 %	0.83 Rf	15.9 AU	5347.7 AU	10.24 %
10	0.83 Rf	16.1 AU	0.88 Rf	65.8 AU	4.89 %	0.92 Rf	4.0 AU	2680.4 AU	5.13 %

3D Chromatogram of 254 nm

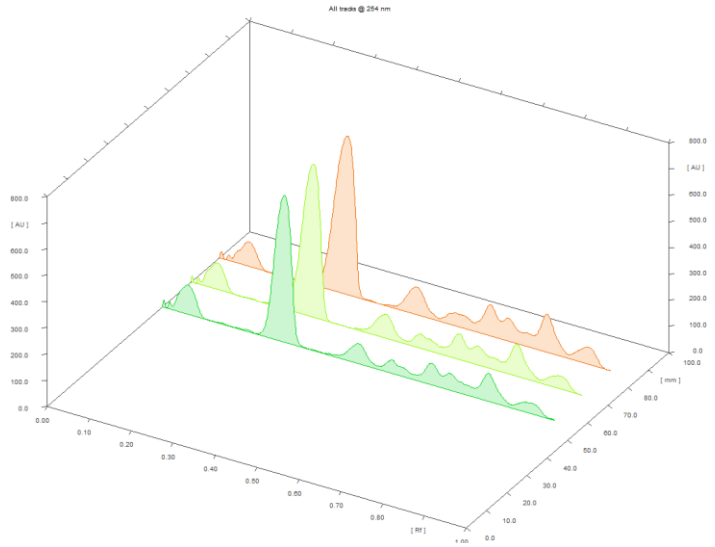


Fig no: 22 shows 3D Chromatogram of 254 nm

Discussion

A qualitative fingerprinting of *Kadukkai Vadagam* has been performed by HPTLC method, which provides qualitative insights into the bioactive Constituents present in the drug. HPTLC shows separation of components present in the Chloroform extract of *Kadukkai Vadagam*. The method may be applied to identify the *Kadukkai Vadagam* from other manufacturing process.

The present study revealed that *Kadukkai Vadagam* showed best results in Toluene: Ethyl Acetate: 9:1 solvent system. After scanning and visualizing the plates in absorbance mode at both 254 nm, 366 nm and visible light range, best results were shown at 254 nm.

TLC plate showed different colour phyto constituents of chloroform extract of *Kadukkai Vadagam*. The bands revealed presence of six greenish, four blue, one dark blue, three bright blue, eight grey, one brown bands showing the presence of glycosides, carbohydrate, phytosterol, alkaloids, phenol, triterpene and saponins.

The results from HPTLC finger print scanned at wave length 254 nm for chloroform extract of *Kadukkai Vadagam*. There are ten polyvalent phyto constituents and corresponding ascending order of Rf values start from 0.05 to 0.89 in which highest concentrations of the phyto constituents was found to be 44.97 % and 10.88 % with its corresponding Rf value were found to be 0.22 and 0.75 respectively.

Bio chemical analysis

Preliminary Basic and Acidic Radical Studies

Table: 14 Results of basic radicals studies

S.NO	Parameter	Result
1	Test for Potassium	+ ve
2	Test For Sodium	+ ve

Interpretation

The basic radical test shows the presence of **Potassium, Sodium** and absence of heavy metals such as lead, arsenic and mercury.

- A sodium and potassium cell-signalling channel plays almost important role in regulatory part of the respiratory system, breathing rhythm, and the body's response to insufficient oxygen levels. So, this drug stimulates normal respiratory mechanism[93].

Results of acid radical studies

Table: 15 shows Results of acid radical studies

S.No	Parameter	Result
1.	Test for Chloride	+ ve
2.	Test for Phosphate	+ ve

Interpretation

The acidic radicals test shows the presence of **Chloride, Phosphate.**

Chloride

- Chloride is needed to keep the proper balancing of body fluids [94]
- It maintains proper blood volume and pressure [95].
- It plays critical roles in inflammatory airway diseases such as Bronchial asthma and Allergic rhinitis [96]

Phosphate

- Phosphate is a charged particle that contains the mineral phosphorus [97]
- The mineral Phosphorus is primarily used for growth and repair of body cells and tissues [98]
- It reduces the histamine release by activated mast cells [99]

Microbial load

Availability of bacterial and fungal load in *Kadukkai Vadagam*

Bacterial and fungal dilutions

Table no: 16 Availability of bacterial and fungal load in *KV*

MICROBES	DILUTION	RESULT
Bacteria	10^{-4}	7
Bacteria	10^{-6}	4
Fungi	10^{-2}	6
Fungi	10^{-3}	4

Discussion

The Herbo-mineral drug are prepared from plant material they are prone to contamination. The contamination of herbal drugs by microorganism not only cause bio deterioration but also reduces the efficacy of drugs.

The toxin produce by microbes makes herbal drugs unfit for human consumption because the contaminated drug may develop unwanted disease instead of disease being cured.

Here the contaminations of *KV* have been examined by bacterial and fungal load.

- Total bacterial load in 10^{-4} dilution is 7 and in 10^{-6} dilution is 4.
- Total fungal load in 10^{-2} dilution is 6 and in 10^{-3} dilution is 4.

Hence the contamination of *KV* is within the WHO norms. Hence, the drug is collected, prepared, stored and packed and decontaminated prior to formulation.

Instrumental analysis

FTIR

Fourier Transform Infra-Red Spectroscopy (FTIR) analysis results in absorption spectra provide information about the functional group and molecular structure of a material.

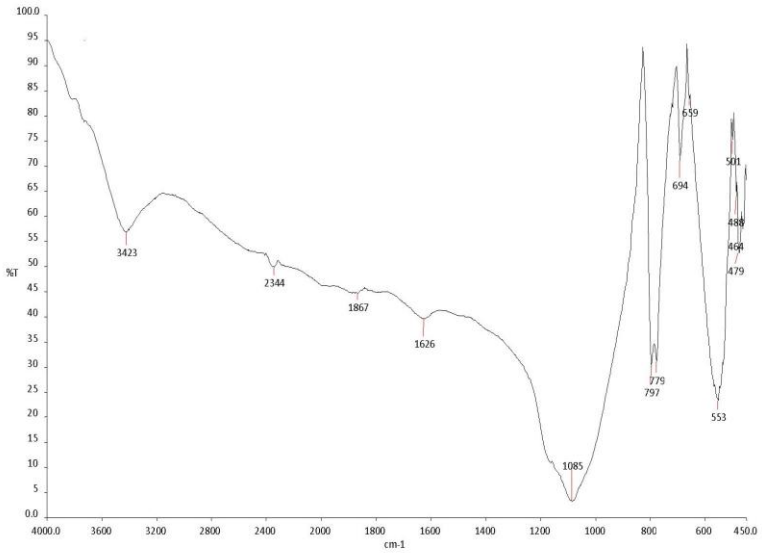


Fig no: 23 shows FTIR Analysis

Table: 17 shows FT-IR Analysis of *Kadukkai Vadagam*

Frequency cm^{-1}	Stretch	Functional group
3423	OH-stretch, H-bonded	Alcohols, Phenols
1626	N-H bond	1° Amines
1085	C-O stretch	Alcohols, Carboxylic acid, Esters, Ethers
779	N-H,C-H=C-H	1°, 2° Amines, Aromatics, Alkenes.
797	N-H,C-H=C-H	1°,2° Amines, Aromatics, Alkenes
694	C-H "oop"	Aromatics.
659	C-H bond	Alkenes
553	C-Br stretch	Alkyl halides
501	C-Br stretch	Alkyl halides

Discussion

FTIR instrumental analysis was done. The test drug was identified to have 9 peaks. They are the functional groups present in the trial drug *Kadukkai Vadagam.*

The above table shows the presence of Alcohol, Phenols, Esters, Amines, Acid, Aromatics, Alkyl halides, Alkene, Ether and Alkanes which are represents the peak value.

- OH group has higher potential towards inhibitory activity against airway inflammation. [100]
- Amines and Carboxylic acids enhances the drug effect against the disease. [101]

SEM (Scanning Electron Microscope with Energy Dispersive X-Ray Analysis)

In addition, the particle size and chemical elements were assessed by Scanning Electron Microscope. SEM is one of the most widely used instrument in research areas.

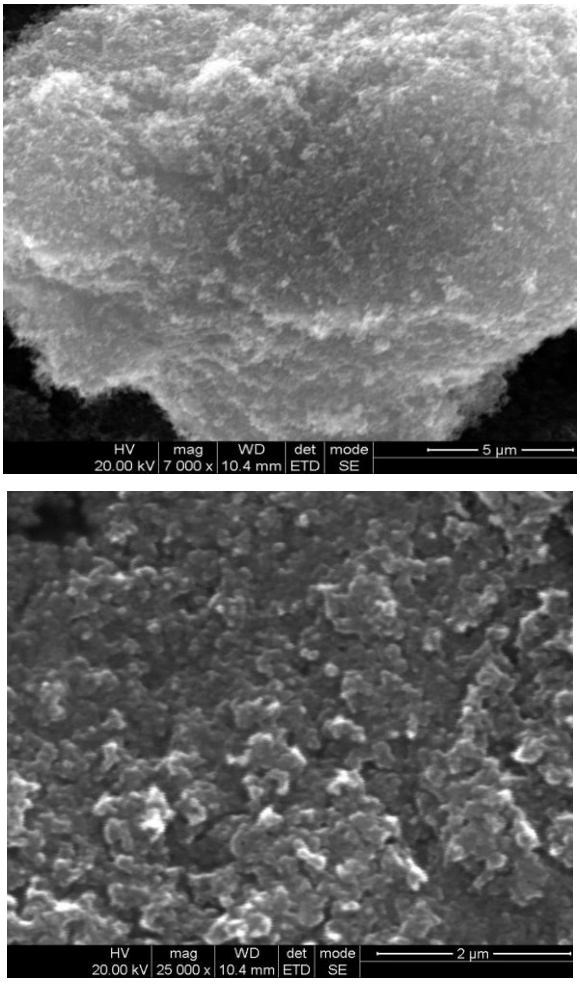


Fig no:24 SEM images shows the partical size of *KV is 2 – 5*µ

Intrepretation

- The SEM reveals the micro size (2 – 5 **μ**) particle of the sample.
- Size and surface of micro particles can be easily manipulated to achieve both passive and active drug targeting.

ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy)

The drug *(Kadukkai Vadagam)* sample was analysed by the Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) to detect the trace elements and other elements quantitatively. The result of ICP-OES is given on the Table no:17.

Table no: 18 ICP-OES of *Kadukkai Vadagam*

S. no	**Elements**	**Wavelength (nm)**	***Kadukkai Vadagam* 100mg**
1.	Al	396.152	BDL
2.	As	188.979	BDL
3.	Cd	228.802	BDL
4.	Cu	327.393	BDL
5.	Hg	253.652	BDL
6.	K	766.491	53.820 mg/L
7.	Na	589.592	04.300 mg/L
8.	Ni	231.604	BDL
9.	Pb	220.353	BDL
10.	P	213.617	225.740 mg/L
11.	Al	396.152	BDL
12.	As	188.979	BDL
13.	Cd	228.802	BDL
14.	Cu	327.393	BDL

BDL : Below Detectable Limit

% = 10000ppm,

1ppm = 1/1000000 or 1ppm = 0.0001%

The toxic metals and the permissible limits

Table no:19 The toxic metals and the permissible limits

Heavy metals	WHO & FDA limits
Arsenic (As)	10 ppm
Mercury (Hg)	1 ppm
Lead (Pb)	10 ppm
Cadmium (Cd)	0.3 ppm

Interpretation

The result indicate that the formulation is extremely safe as it contains heavy metals below detectable limits.

ICP-OES reveals high concentration of P in *Kadukkai Vadagam*. It also possess Na and K. Phosphorous is also used in treating asthma with symptoms of accumulation of mucus in the bronchi, dyspnoea and the sensation of heat in the chest, aetiology being temperature changes in atmosphere. Bichromate form of potassium relieves hyper production of mucus. [102]

XRD

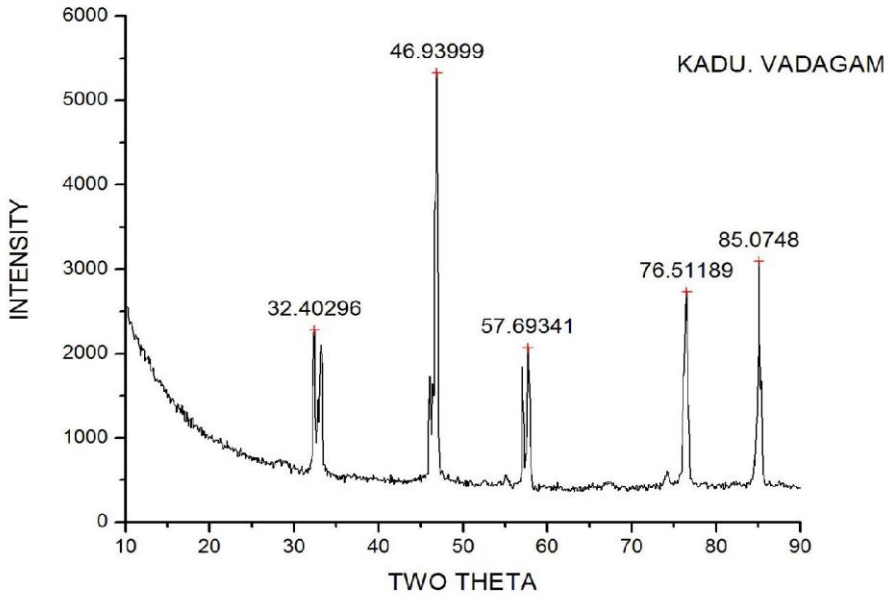


Fig.no:25 XRD images of *KV*

Interpretation

The structure, the size and shape of the particles are highly dependent on the route of synthesis and high lights the efficacy of the drug. The micro particles may enhance bio absorbtion of the drug.

The major diffraction peaks are identified after XRD analysis is *KV* concluded that range is 45-60 nm is association with organic molecules probably place an important role in making it biocompatible and nontoxic at therapeutic doses. Other elements present in *KV* act has additional supplement and possibly helps in increases the efficacy of the formulation.

Toxicity studies

Acute oral toxicity study of Kadukkai Vadagam

Table: 20 Dose finding experiment and its behavioural signs of acute oral Toxicity Observation

S.No	Physical Parameters	Observation	
		Control group	Test group
1	Body weight	Normal	Normally increased
2	Assessments of posture	Normal	Normal
3	Signs of Convulsion Limb paralysis	Normal	Absence of sign (-)
4	Body tone	Normal	Normal
5	Lacrimation	Normal	Absence
6	Salivation	Normal	Absence
7	Change in skin color	No significant color change	No significant color change
8	Piloerection	Normal	Normal
9	Defecation	Normal	Normal
10	Sensitivity response	Normal	Normal
11	Locomotion	Normal	Normal
12	Muscle gripness	Normal	Normal
13	Rearing	Mild	Mild
14	Urination	Normal	Normal

Table : 21 (Observational study Results)

No	Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	**Control**	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	**2000 mg/kg**	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

1. Alertness 2. Aggressiveness 3. Pile erection 4. Grooming 5. Gripping
6. Touch Response 7. Decreased Motor Activity 8. Tremors 9. Convulsions
10. Muscle Spasm 11. Catatonia 12. Muscle relaxant 13. Hypnosis 14. Analgesia
15. Lacrimation 16. Exophthalmos 17. Diarrhoea 18. Writhing 19. Respiration
20. Mortality. (+ Present, - Absent)

Table: 22(Body weight Observation)

DOSE	**DAYS**		
	1	7	14
CONTROL	166.6 ± 1.95	168.2 ± 4.82	169.2 ± 3.12
2000 mg/kg	172.3 ± 2.18	174.2 ± 1.26	177.2 ± 3.27

Table: 23 (Water intake (ml/day) of Wistar albino rats group exposed to *K V*

DOSE	**DAYS**		
	1	7	14
CONTROL	32.5 ± 1.34	33.0 ± 10.13	32.4 ± 3.13
2000 mg/kg	30.4 ± 2.33	33.6 ± 1.91	35.9 ± 2.19

N.S- Not Significant,**($p > 0.01$), *($p > 0.05$), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Table: 24 Food intake (gm/day) of Wistar albino rats group exposed to *K V*

DOSE	DAYS		
	1	7	14
CONTROL	25.16 ± 6.36	27.60 ± 3.12	27.61 ± 5.46
2000 mg/kg	26.42 ± 1.64	27.31 ± 3.22	28.12 ± 6.14

Discussion

The acute toxicity result shows no mortality rate up to 2000mg/body weight per kg. It showed changes in alertness, grooming, touch response. The behavioural changes are normal. Hence the test drug *Kadukkai Vadagam* is a safe herbal drug and can be used for long time administration.

Repeated Dose 28- days oral toxicity study of *Kadukkai Vadagam*

Table: 25 Body weight of wistar albino rats group exposed to *KV*

DOSE	DAYS				
	1	7	14	21	28
CONTROL	172.0 ± 4.23	172.4 ± 3.42	174.7 ± 1.36	174.2 ± 1.33	175.7 ± 1.31
LOW DOSE	181.2 ± 3.12	182.7 ± 4.64	185.4 ± 3.18	185.8 ± 1.86	186.12 ± 2.36
MID DOSE	178.6 ± 1.34	179.3 ± 2.14	180.4 ± 6.32	182.1 ± 3.16	183.7 ± 3.12
HIGH DOSE	177.4 ± 8.14	179.6 ± 3.12	179.6 ± 2.16	180.0 ± 6.21	181.92 ± 2.19

NS- Not Significant, **($p > 0.01$),*($p > 0.05$), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Table: 26 Water intake (ml/day) of Wistar albino rats group exposed to *K V*

DOSE	DAYS				
	1	6	14	21	28
Control	51.5 ± 7.15	50.0 ± 8.23	58.5 ± 6.63	49.12 ± 7.19	51.5 ± 3.96
Low dose	38.5 ± 3.41	39.4 ± 3.62	39.27 ± 4.12	38.2 ± 3.29	39.9 ± 3.13
Mid dose	36.7 ± 4.13	36.3 ± 2.21	37.1 ± 4.13	38.4 ± 6.31	38.4 ± 3.34
High dose	32.1 ± 1.32	33.2 ± 4.13	34.7 ± 3.13	32.2 ± 1.73	40.4 ± 2.65

N.S- Not Significant, **($p > 0.01$), *($p > 0.05$), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Table: 27 Food intake (gm/day) of Wistar albino rats group exposed to *KV*

DOSE	DAYS				
	1	7	14	21	28
CONTROL	29.12 ± 5.37	28.5 ± 4.22	29.5 ± 4.27	32.5 ± 3.87	33.12 ± 6.32
LOW DOSE	33.7 ± 4.98	34.3 ± 1.22	33.1 ± 6.18	35.4 ± 6.12	35.6 ± 2.12
MID DOSE	32.2 ± 4.75	33.2 ± 6.80	37.2 ± 1.25	33.4 ± 2.68	32.2 ± 1.44
HIGH DOSE	32.2 ± 2.34	32.2 ± 2.64	34.6 ± 2.16	38.2 ± 3.14	37.0 ± 1.62

N.S- Not Significant, **($p > 0.01$), *($p > 0.05$), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test

Table: 28 Haematological parameters of Wistar albino rats group exposed to *KV*

Category	Control	Low dose	Mid dose	High dose
HB (g/dl)	14.8 ± 1.88	13.1 ± 3.16	13.64 ± 3.66	14.28 ± 0.96
Total WBC($\times 10^3$ l)	10.91 ± 2.59	10.25 ± 6.73	11.28 ± 2.31	11.40 ± 6.14
Neutrophils (%)	32.65 ± 1.06	32.13 ± 4.14	33.11 ± 1.46	34.40 ± 3.20
Category	Control	Low dose	Mid dose	High dose
lymphocyte (%)	69.34 ± 2.48	70.16 ± 6.12	71.58 ± 4.66	74.13 ± 4.16
Monocyte (%)	0.78 ± 0.17	0.76 ± 0.04	0.80 ± 0.13	0.83 ± 0.36
Eosinohil (%)	0.64 ± 0.09	0.61 ± 0.16	0.58 ± 0.43	0.73 ± 0.14
Platelets cells10^3/µl	687.17 ± 8.76	722.71 ± 2.16	705.18 ± 2.0	735.16 ± 3.14
Total RBC 10^6/µl	7.99 ± 0.12	6.82 ± 1.37	7.12 ± 1.89	7.18 ±7.72
PCV%	37.79 ± 0.6	41.35 ± 8.13	42.18 ± 1.68	43.82 ± 2.54
MCHC g/Dl	33.6 ± 2.23	32.19 ± 5.29	33.18 ± 4.22	32.93 ± 1.24
MCV fL(µm^3)	49.17 ± 3.64	48.29 ± 1.22	50.18 ± 1.24	50.94 ± 1.44

N.S- Not Significant, **($p > 0.01$), *($p > 0.05$), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Table: 29 Biochemical Parameters of Wistar albino rats group exposed to *KV*

BIOCHEMICAL PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
GLUCOSE (R) (mg/dl)	76.45 ± 13.4	76.16 ± 2.34	75.26 ± 2.20	77.42 ± 2.64
T.CHOLOSTEROL (mg/dl)	115.26 ±1.83	109.45 ± 4.13	118.42 ± 4.78	123.22 ± 3.73
TRIGLY(mg/dl)	46.35 ± 1.48	44.22 ± 1.48	48.58 ± 1.30	47.66 ± 3.33
LDL	72.81 ± 2.13	76.24 ± 8.14	74.8 ± 2.14	70.64 ± 4.32
VLDL	15.2 ± 2.44	14.42 ± 4.64	14.04 ± 1.64	13.94 ± 1.46
HDL	26.66 ± 6.88	23.86 ± 6.24	26.10 ± 2.66	30.68 ± 1.12
Ratio1 (T.CHO/HDL)	4.42 ± 2.44	4.46 ± 3.14	4.64 ± 2.14	4.18 ± 2.12
Ratio2 (LDL/HDL)	2.83 ± 4.22	2.14 ± 2.22	2.28 ± 2.20	2.16 ± 6.22
Albumin(g/dl)	3.63 ± 0.17	3.18 ± 0.42	3.16 ± 2.62	2.94 ± 4.16

NS- Not Significant,**($p > 0.01$), * ($p > 0.05$), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Table: 30 Renal function test of Wistar albino rats group exposed to *KV*

PARAMETERS (mg/dl)	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
UREA	13.35 ± 0.99	13.14 ± 0.16	12.96 ± 1.98	12.28 ± 3.62
CREATININE	0.28 ± 0.08	0.36 ± 0.06	0.52 ± 0.04	0.66 ± 0.02
BUN	15.02 ± 0.10	14.28 ± 1.92	14.09 ± 1.34	14.02 ± 4.32
URIC ACID	5.17 ± 0.35	5.01 ± 1.03	5.12 ± 3.15	4.58 ± 1.33

NS- Not Significant, **(p > 0.01), * (p >0.05) , n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Table: 31 Liver Function Test of of Wistar albino rats group exposed to *Kadukkai Vadagam*

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
T BILIRUBIN (mg/dl)	0.48 ± 0.07	0.40 ± 1.26	0.41 ± 3.28	0.39 ±1.25
SGOT/AST(U/L)	79.95 ± 1.39	76.15 ± 1.31	77.31 ± 3.03	79.25 ± 4.03
SGPT/ALT(U/L)	31.23 ± 1.28	32.91 ± 3.59	36.24 ± 7.48	34.12 ± 1.68
ALP(U/L)	143.25 ± 8.70	146.12 ± 1.37	143.16 ± 4.17	145.33 ± 1.65
T.PROTEIN(g/dL)	5.32 ± 0.38	5. 22 ± 1.14	6.01 ± 3.23	6.93 ± 1.46

NS- Not Significant, **(p > 0.01), * (p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Discussion

The dose selected for the repeated oral toxicity study was 100mg, 200mg/kg of *Kadukkai Vadagam*

All the animals were free of intoxicating signs throughout the dosing period of 28 days. No physical changes were observed throughout the dosing period. No mortality was observed during the whole experiment. No abnormal deviations were observed. No significant changes were observed in the values of different parameters studied when compared with controls and values obtained were within normal biological and laboratory limits.

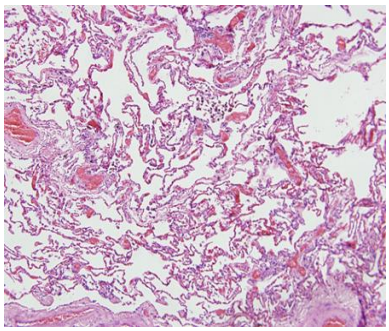
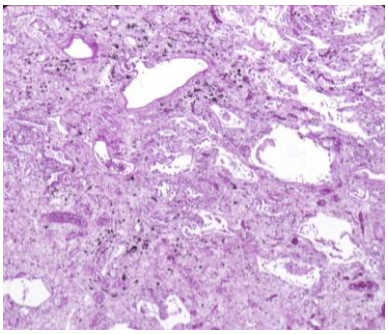
The weights of organs and body weight recorded did not show any significant differences in the treatment and the control group indicating that *Kadukkai Vadagam* was not toxic to kidney, liver and spleen.

There was no significant changes were observed in haemoglobin (Hb), Red blood cell (RBC), White blood cell (WBC), Packed cell volume (PCV), Erythrocyte sedimentation rate (ESR) in all the treated groups as compared to respective control groups.

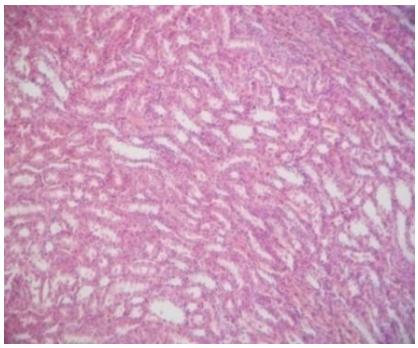
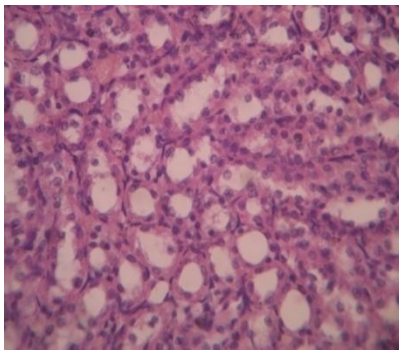
Repeated Oral Toxicity Study Histo pathology

Control Kadukkai Vadagam(300 mg/kg)

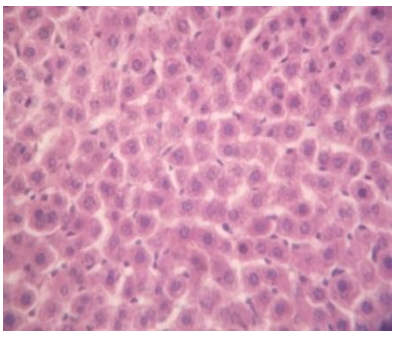
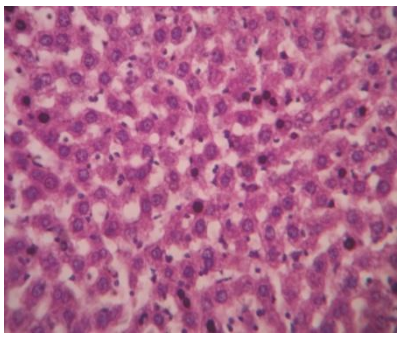
Lungs

Kidney

Liver

Spleen

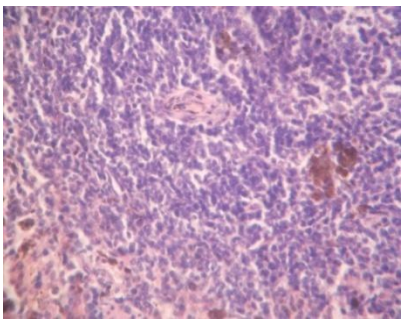
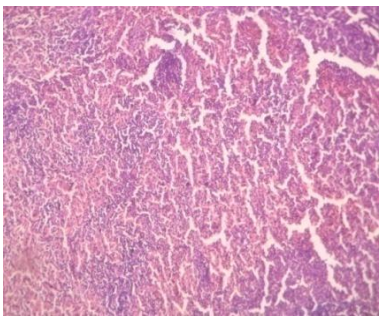



Fig no: 26 shows Histopathology slides.

Interpretation

The above slides show the histopathology studies of repeated oral toxicity study. There is no toxicological abnormality seen in the vital organs after administration of the test drug *Kadukkai Vadagam.* Thus the safety of the drug is revealed so that it can be administered for long time without any side effects.

Pharmacological study

Bronchodilator activity

Effect of Histamine induced bronchoconstriction in guinea pig:

Table No: 32 shows Histamine induced bronchoconstriction in guinea pig

Serial No	Group	Onset of Convulsion in sec.	% protection
1	Control	93.75 ± 0.39	--
2	Standard (Chlopheniramine maleate)	992.51 ± 0.53***	100
3	*KV* (200mg/kg)	226.58 ± 0.94*	24
4	*KV* (400mg/kg)	549.85 ± 0.62**	57

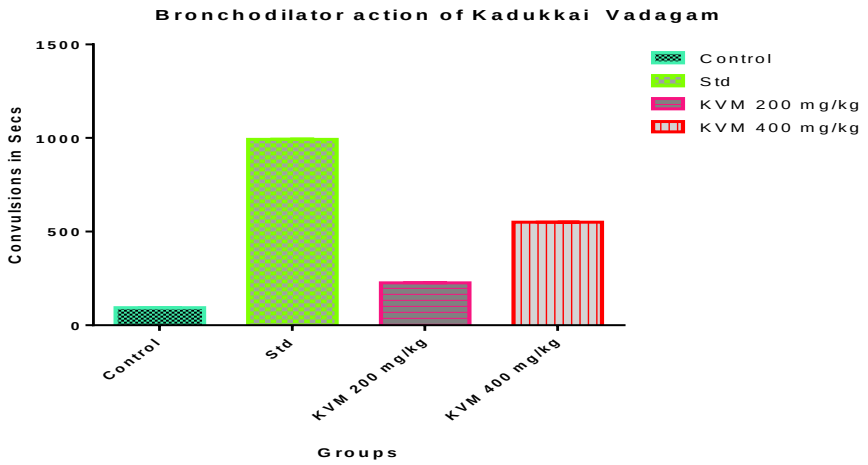


Chart: 1 shows Bronchodilator action of Kadukkai Vadagam

Discussion

From the above results, we can confirm that *Kadukkai Vadagam* possesses bronchodilator activity nearer to fifty percentage when compared with chlopheniramine maleate as a standard drug.

Anti – histamine activity

Table: 33 Effect of *Kadukkai Vadagam* isolated guinea pig ileum preparation.

S.No	Low dose Histamine (100 µg/ml)	Percent of maximum response	
		Histamine alone	Histamine + *KV* (1mg/ml)
1.	0.20	7.58 ± 0.30	7.38 ± 0.30
2.	0.26	13.2 ± 0.3	9.01 ± 0.03**
3.	0.36	22.48 ± 0.60	17.40 ± 0.31***
4.	0.66	45.27 ± 0.36	34.24 ± 0.59***
5.	1.2	91.93 ± 0.45	66.01 ± 0.91***

Values are expressed in mean ± SEM, *p< 0.05; **p< 0.01, ***p<0.001compared with histamine alone (45mm as 100%); n=3.

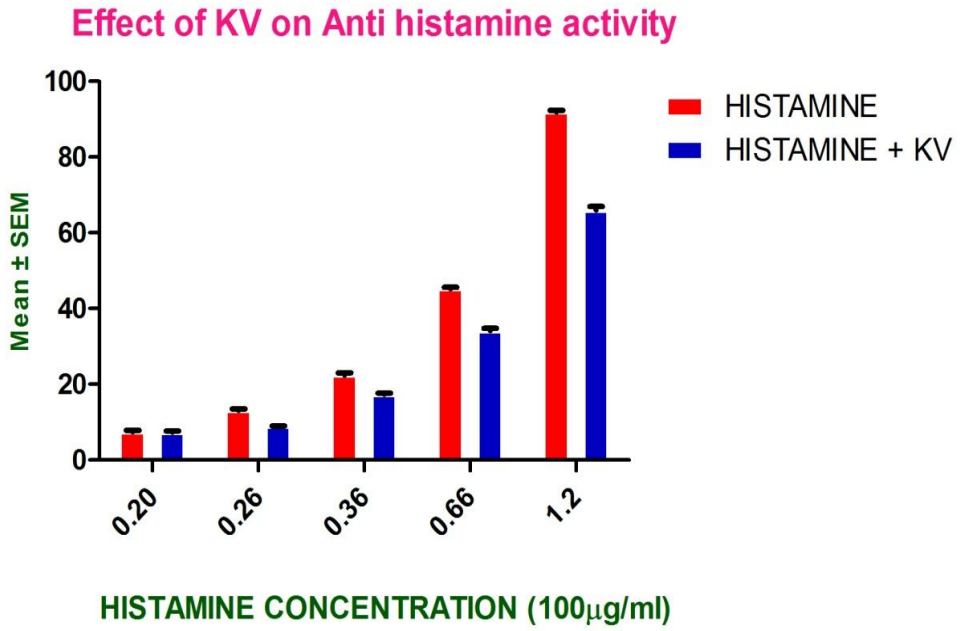


Chart: 2 shows Anti histamine activity

Discussion

Mediators like histamine, serotonin, and acetylcholine are implicated in various ways in the pathogenesis of Asthma. Histamine is the most implicating mediator in broncho constriction that accompany asthma although the role of serotonin in asthma is uncertain. *KV* inhibited the histamine induced bronchospasm (vascular permeability) in rats, when compare with cetrizine as standard. Here, *KV* possesses the Anti-histamine activity.

Anti oxidant activity

Table: 34 DPPH Assay of *KV*

Sample concentration (µg/ml)	Absorbance		Percentage of Inhibition	
	Drug	Standard	Drug	Standard
Control	0.5461	0.324	-	-
1.25	0.4822	0.254	11.701	21.60
2.50	0.3611	0.127	33.876	60.80
5	0.2455	0.111	55.044	65.74
10	0.1320	0.062	75.828	80.86
20	0.1123	0.021	79.436	93.51

*****µg/ml: microgram per millilitre. Drug: Kv(1.25-20µg/µl). Standard: Ascorbic acid(10mg/mlDMSO)*

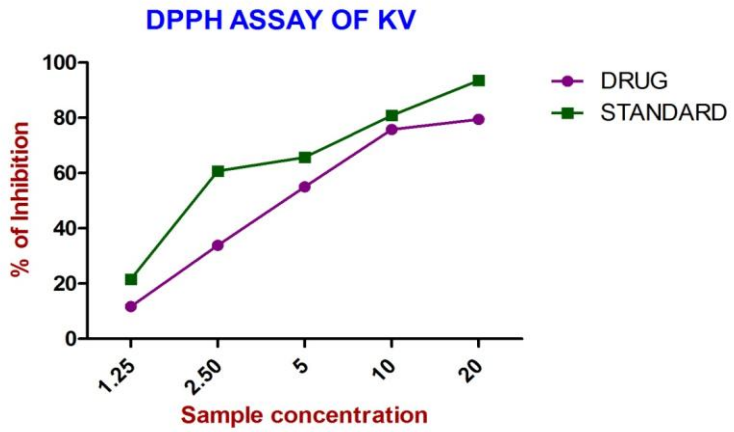


Chart: 3 shows Anti-oxidant activity

Discussion

Anti oxidants can compensate the oxidative stress that correlates with asthma, can reduce the symptoms of asthma and improve pulmonary functions.

Oxidative stress resulting from a increased amount of reactive oxygen speices and an imbalance between oxidants and anti oxidants plays an important role in the pathogenesis of asthma.[103]

6. CONCLUSION

The trial drug ***"Kadukkai Vadagam"*** was taken from the Siddha literature **"*Athmaratchamirtha Vaidhiya Saara Sangiragam* (part 2)''** to evaluate the safety and efficacy of the drug in *Swasakasam* (Bronchial asthma).

The ingredients of the trial drug was identified and authenticated by the experts of *Gunapadam*. By the classical methods, ingredients of the trial drug *Kadukkai Vadagam* was purified and the drug was prepared. In the purification process toxins was eliminated and increases its efficacy. In the grinding process of this drug particle size of the drug became into nana particle for its better bio availability.

Phytochemical analysis of the drug shows presences of glycosides, saponins, carbohydrates, Phytosterol, Phenol, Triterpenes and Protein. A synergistic effect of all these Glycosides, Saponin, Carbohydrate, Phytosterol, Phenol, Triterpenes and Protein increases the potency of the drug against Bronchial asthma.

Biochemical analysis of basic radicals confirms the presence of Potassium and Sodium. A sodium and potassium cell-signalling channel plays almost important role in regulatory part of the respiratory system, breathing rhythm, and the body's response to insufficient oxygen levels. So this drug stimulates normal respiratory mechanism.

Biochemical analysis of acid radicals shows the presence of Chloride, Phosphate. Chloride plays critical roles in inflammatory airway diseases such as Bronchial asthma.

Instrumental analysis FT-IR results shows presence of Alcohol, Phenols, Esters, Amines, Acid, Aromatics, Alkyl halides, Alkene, Ether and alkanes groups. Alcohol group has anti asthmatic effect. It has higher potential towards inhibitory activity against airway inflammation.

SEM picture shows micro particle sizes. It represents the drug is more absorbable and easily to reach the cell.

ICP-OES results show the presence of Phosphorus, Sodium, and Potassium. Phosphorus is best suited for cough that occurs with asthma. So it is indicated in the treatment of bronchial asthma.

Kadukkai Vadagam did not produce any oral acute or sub-acute toxicity in rats. So the drug is non-toxic and safe. The histopathology studies of acute and sub-

acute toxicity shows that there is no toxicological abnormality seen in the vital organs after administration of the test drug *Kadukkai Vadagam*.

Kadukkai Vadagam could be conformed as No-Observed-Adverse Effect Level (NOAEL) drug as it acts harmless under normal usage and to be of no toxicological concern.

After evaluate the safety the drug, the Bronchodilator and anti-histaminic property of *Kadukkai Vadagam* is elaborated. So it can be concluded that this drug inhibits the tone of tracheal and bronchial muscles and thus has a bronchodilator action. It is possible that anti-histamine activity of the *Kadukkai Vadagam* mainly involves inhibiting the histamine induced bronchospasm. The *KV* extract has more or less equal DPPH scavenging activity when compared to the standard. The *KV* extract has a marked antioxidant activity at higher concentrations.

From the above scientific evaluation, the author concludes that the drug *Kadukkai Vadagam* is proficient with the new hope in the treatment of Bronchial asthma which is cost effective and has fair preparation method.

7. Summary

In our *Siddha* literature symptoms of the *Swasa kasam* was correlated with bronchial asthma. Generally due to the imbalance of the *prana vayu*, three humours in the body gets vitiated, then it leads to broncho constriction.

Hence the author conducts the detailed scientific validation of *Kadukkai Vadagam* for Bronchodilator activity, Anti-oxidant activity and Anti-histamine activity.

The study was accepted by Institutional screening committee and then approved by the Institutional Animal Ethical Committee (IAEC).

In this study, various types of analyzing studies for efficacy and toxicity were conducted in the SCRI and Baid Metha College.

In the Organoleptic studies *KV* has dark brown colour, sour in taste, hard in touch, 1cm in size and slightly soluble in water.

In the Physiochemical analysis studies, *KV* has contains Total ash 15.12%, Water soluble ash 3.13%, Acid insoluble ash 0.68%, Water soluble extractive 44.00%, Alcohol soluble extractive 45.81%.

The phytochemical analysis reveals the presence of Glycosides, Saponin, Carbohydrate, Phytosterol, Phenol, Triterpenes and Protein which act as a defence system against diseases and more accurately to protect against swasa kasam diseases.

In HPTLC analysis finger print scanned at wave length 254 nm for chloroform extract of *KV*. There are ten polyvalent phyto constituents and corresponding ascending order of Rf values start from 0.05 to 0.89 in which highest concentrations of the phyto constituents was found to be 44.97 % and 10.88 % with its corresponding Rf value were found to be 0.22 and 0.75 respectively.

In the Bio-Chemical analysis of the *KV* drug contains Sodium, Potassium which stimulates normal respiratory mechanism in bronchial asthma.

In the examination of *KV* drug, bacterial and fungal load has within the WHO norms. Hence the drug KV is safe.

In the FTIR instrumental analysis, the drug *KV* contains presence of Alcohol, Phenols, Esters, Amines, Acid, Aromatics, Alkyl halides, Alkene, Ether and alkanes which are represents the peak value. OH group has higher potential towards inhibitory activity against airway inflammation. Amines and Carboxylic acids possess which enhances the drug effect against the disease.

In the SEM Analysis, *KV* drug contains the micro size particle of the sample.

ICP-OES results indicate that the formulation is extremely safe as it contains heavy metals below detectable limits.

XRD pattern of the trail drug *KV* shows some good crystalinity. The major diffraction peaks are identified after XRD analysis

The acute toxicity result shows no mortality rate up to 2000mg/kg. It showed changes in alertness, grooming, touch response. The behavioral changes are normal. Hence the test drug *KV* is a safe herbal drug and can be used for long time administration.

There is no toxicological abnormality seen in the vital organs after administration of the test drug *KV*. Thus the safety of the drug is revealed so that it can be administered for long time without any side effects.

In the Pharmacological study *KV* possesses bronchodilator activity, Anti-histamine activity and Anti oxidants. Hence It can reduce the sympotoms of asthma and improve pulmonary funtions.

An incredible action of this drug value against the disease of Bronchial asthma has been revealed from this study of. *KV*. This must be implicated in the future clinical studies and preclinical chronic toxicity studies.

8. FUTURE SCOPE

The trial drug ***Kadukkai Vadagam*** has its own potency in treating Bronchial asthma in animal model which has been established in this study. An incredible action of this drug value against the disease of Bronchial asthma has been revealed from this study of ***Kadukkai Vadagam***. This must be implicated in the future clinical studies and preclinical chronic toxicity studies.

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INGREDIENTS OF KADUKAI VADAGAM

Fig.no:1*Terminalia chebula*

Fig.no:*2 Black salt*

Fig.no:3 *Cyperus rotandus*

Fig.no:*4.Hyoscyamus niger*

Fig.no: 5 *Zingiber officinale)*

Fig.no:6 *Plumbago indica*

INGREDIENTS OF KADUKAI VADAGAM

Fig no:7 *Piper longum*

Fig.no:8 *Piper nigrum*

Fig.no: 9 *Piper longum*

Fig.no: 10 *Piper nigrum*

Fig no: 11 Rock salt

Fig no:12*Carum copticum*

PREPARAION OF THE KADUKKAI VADAGAM

Fig no: 13 Adding zinger juice, lemon juice and butter milk finally in grinding process

Kadukkai vadagam

Fig no:14

The Tamil Nadu Dr.M.G.R.Medical University

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This Certificate is awarded to Dr/Mr/Mrs.....A. Kalpana.....

for participating as ~~Resource~~ ~~Person~~ / Delegate in the Eighteenth Workshop on

" RESEARCH METHODOLOGY & BIOSTATISTICS "

FOR AYUSH POST GRADUATES & RESEARCHERS

Organized by the Department of Siddha

The Tamil Nadu Dr. M.G.R. Medical University from 20 th to 24 th July 2015.

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VICE - CHANCELLOR

C.L.BAID METHA COLLEGE OF PHARMACY

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CERTIFICATE

This is to certify that the project entitled, **Toxicological and Pharmacological study on KADUKKAI VADAGAM & ORITHAZH THAMARAI (*Ionidium suffrutiocosum*) CHOORANAM** in rats submitted in partial fulfilment for the degree of **M.D. (siddha)** was carried out at C.L. Baid Metha college of Pharmacy, Chennai-97, in the Department of Pharmacology during the academic year of 2016-2017. It has been approved by the **IAEC**

No: IAEC/XLVIII/07/CLBMCP/2016

C.L. Baid Metha College of Pharmacy CHENNAI-97 Thoraipakkam

(Dr.P.Muralidharan)

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